This is a digital copy of a book that was preserved for generations on library shelves before it was carefully scanned by Google as part of a project to make the world's books discoverable online.

It has survived long enough for the copyright to expire and the book to enter the public domain. A public domain book is one that was never subject to copyright or whose legal copyright term has expired. Whether a book is in the public domain may vary country to country. Public domain books are our gateways to the past, representing a wealth of history, culture and knowledge that's often difficult to discover.

Marks, notations and other marginalia present in the original volume will appear in this file - a reminder of this book's long journey from the publisher to a library and finally to you.

Usage guidelines

Google is proud to partner with libraries to digitize public domain materials and make them widely accessible. Public domain books belong to the public and we are merely their custodians. Nevertheless, this work is expensive, so in order to keep providing this resource, we have taken steps to prevent abuse by commercial parties, including placing technical restrictions on automated querying.

We also ask that you:

+ *Make non-commercial use of the files* We designed Google Book Search for use by individuals, and we request that you use these files for personal, non-commercial purposes.

+ *Refrain from automated querying* Do not send automated queries of any sort to Google's system: If you are conducting research on machine translation, optical character recognition or other areas where access to a large amount of text is helpful, please contact us. We encourage the use of public domain materials for these purposes and may be able to help.

+ *Maintain attribution* The Google "watermark" you see on each file is essential for informing people about this project and helping them find additional materials through Google Book Search. Please do not remove it.

+ *Keep it legal* Whatever your use, remember that you are responsible for ensuring that what you are doing is legal. Do not assume that just because we believe a book is in the public domain for users in the United States, that the work is also in the public domain for users in other countries. Whether a book is still in copyright varies from country to country, and we can't offer guidance on whether any specific use of any specific book is allowed. Please do not assume that a book's appearance in Google Book Search means it can be used in any manner anywhere in the world. Copyright infringement liability can be quite severe.

About Google Book Search

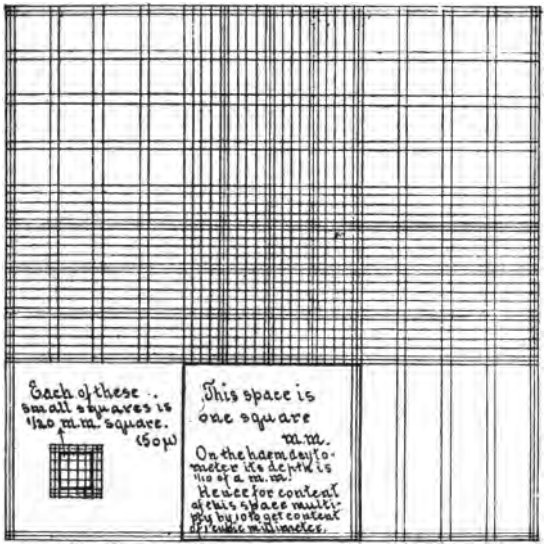
Google's mission is to organize the world's information and to make it universally accessible and useful. Google Book Search helps readers discover the world's books while helping authors and publishers reach new audiences. You can search through the full text of this book on the web at `http://books.google.com/`

COUNTWAY LIBRARY
HC 1H95 I

Disease and cause	Incubation period	Source of infection	Mode of transmission	Period of communicability	Laboratory diagnosis	Salient clinical features	Methods of control and remarks
1. Cerebrospinal [illegible]	2 to 10 days (commonly 7).	Naso-pharyngeal discharges.	Contact, droplet infection from sneezing or coughing of carriers or cases. Well carriers particularly dangerous to children.	So long as meningococcus recovered from [illegible] rior naso-p "Persistent riers (3 mo longer) mc mon thar erly suppo	Cultures from pharynx of carrier or spinal [illegible]	Rapid onset with marked headache, stiffness of neck and vomiting. Cutaneous	Isolation of carrier or case until pharyngeal cultures negative 2 to 4 times. Case
[illegible]	[illegible] to 21 ys.	Skin and mucous membrane lesions.	Contact with patient or articles soiled by discharges of lesions.	Until pri scabs ha appeared skin and r lesions.			
3. Diphtheria. B. diphtheriæ.	2 to 5 days. Longer when carrier stage precedes case.	Throat, nose, vaginal and wound surface discharges. "Persistent" carriers often show virulent facilli in tonsillar crypts.	Contact with discharges from case or carrier. Fomites or through milk or milk products.	Until bac appear fr charges o Healthy may carr tion for m			
4. German Measles (Rotheln or rubella). Cause unknown.	10 to 21 days.	Oral and possibly nasal secretions.	Direct contact with patient or fresh oral secretions.	Eight ds onset of	[illegible] creased at end of disease.	pears on face. Disappears in 2 or 3 days. Course mild. Presence hybrid rash-morbilliform on face, scarlatinal inner surface thigh, characteristic.	3 weeks. No quarantine. Eruption most marked about mouth and nose. Extends to trunk and extremities by crops. Throat condition and swelling of post neck glands may simulate scarlatina. Drug, ptomaine and enema eruptions may simulate.

Harvard University
Library of
The Medical School
The Gift of
Nu Sigma Nu Fraternity
ACADEMIA HARVARDIANA IN NOVO ANO ECCLESIAE CHRISTO VERI ET TAS

(Morbilli). Filterable virus.	days (usually 14).	secretions.	with patient or oral or nasal secretions.	nasal mucous membranes involved. 2 days before and 5 days after rash.	crease of lymphocytes before appearance of Koplik spots.	marked fever and malaise. Koplik spots on 1st or 2d day. Characteristic rash about 4th day, first on face. Crescent grouping. Face appears swollen. Conjunctivitis and bronchitis common.	of communicability. Quarantine children exposed to infection from school and public gatherings 14 days. Isolate contacts showing 1° F. rise in temperature. No immunization. Disinfect articles soiled with secretions of nose and throat.
6. Mumps. Epidemic parotitis.	4 to 25 days. Usually 14 days, common maximal is 21 days.	Secretions of mouth and possibly nose.	Direct contact with case or articles freshly soiled with secretions of mouth or, possibly, nose.	Unknown but probably until parotid gland returns to normal.	Relative increase in mononuclears of blood.	Pain on opening mouth, chewing or swallowing. Parotid enlargement front and below ear of one or both sides. At times other salivary glands involved. Stomatitis.	Isolate patient for period of presumed infectivity. Quarantine exposed children from school or public gatherings at least 14 days. No immunization. In early diagnosis look for inflammation of Steno's duct. Consider metastatic orchitis.
7. Poliomyelitis. (Heine-Medin disease). Filterable virus. Cultured by Noguchi.	3 to 10 days (commonly 6 days).	Nose, throat, and bowel discharges of case or articles freshly soiled thereby.	Direct contact with case or carrier. Indirectly by articles freshly soiled with virus.	Not known. Apparently not more than 21 days from onset of disease.	Usually slight leukopenia. Occasionally moderate leukocytosis. Sp. fluid clear, moderate lymphocytosis, increased protein and pressure.	Onset may resemble common febrile diseases of childhood or meningitis. Fully developed flaccid paralysis, most often lower extremities (75%) comes on from 1 to 7 days.	Isolation of cases in screened room. Quarantine of exposed children, also of adults who deal with children for 14 days from exposure to case. Disinfect nose, throat and bowel discharges. Healthy carriers supposed to be common.
8. Scarlet Fever (Scarlatina). Cause unknown.	2 to 7 days (usually 3 to 4 days).	Nose, throat and ear discharges and from broken-down glands.	Direct contact with case or articles soiled by infectious discharges or by contaminated milk.	Four weeks from onset of disease and until all abnormal discharges and open sores have ceased.	Marked leukocytosis in severe cases. May be some eosinophilia. Examine urine for albumen.	Sudden onset with vomiting, tendency to high fever, tachycardia, angina and enlarged glands at angle of jaw. Boiled-lobster-like eruption by end of 24 hours appearing first on neck and upper chest. Most marked about armpits and region of Scarpa's triangle. Desquamation often in sheets.	Isolate case 5 or 6 weeks and quarantine contacts for 5 days. Disinfect all discharges. Pasteurize milk. Consider possibility of drug or serum rashes. Look for strawberry tongue. Angina may be membranous.
9. Smallpox. (Variola). Cause unknown. Possibly a filterable virus. Possibly Cytorrhyctes variolæ.	12 to 14 days. For variola inoculata, 7 to 8 days.	Lesions of skin and mucous membranes.	Personal contact, also by articles soiled with discharges. Flies may transfer virus.	From first symptoms to disappearance of scabs and crusts.	Reduction of polynuclears with increase of lymphocytes until pustule formation—then leukocytosis from mixed (pyogenic) infection. Inoculation rabbit's cornea gives Guarnieri bodies in 3 days. Immediate vaccination reaction (24 to 48 hours) shows immunity to smallpox.	High fever, marked headache, backache, and vomiting for 3 days. Rarely initial scarlatinal or measly rashes on 2d day. Shotty papular rash at hair line and wrists on 4th day with fall of fever. Eruption on trunk and extremities follows facial one in 24 hours. A remittent fever accompanies the pustular stage. The pustules are markedly umbilicated.	General vaccination in infancy. Revaccination at school entry or in times of unusual prevalence. Hospital isolation in screened wards. Disinfection of all discharges or soiled articles. Segregation of all contacts 14 days or until protected by vaccination. Multilocular vesicles on 6th day. Umbilicated pustules, with rise of fever on 8th day. Same type lesion on same region.
10. Whooping cough (Pertussis). Bordet-gou bacillus.	Within 14 days.	Discharges from bronchial or laryngeal mucous membranes of cases.	Direct contact with cases or articles freshly soiled by discharges.	Particularly contagious in early stages before characteristic whoop. Communicability probably only lasts 2 weeks after "whoop."	Primary leukocytosis, then lymphocytosis, then eosinophilia. Examine smear from sputum of early stages and culture same.	Onset with coryza or bronchitis for 3 to 14 days before development of characteristic "whoop."	Isolate child 2 weeks after "whoop." Disinfect discharges. Cats and dogs susceptible and may transmit disease. Vaccination possibly of prophylactic value.



The Ruling of the Turck Disc. Explanation of the Squares.—In the first place, we have the square which encloses the entire ruled surface. This is made up of nine squares, each 1 mm. square. These are the squares to use in connection with leukocyte counts with the white pipette. They may be termed the large squares. The very smallest squares which can be found are those made by the intersection of the triple ruled lines in the center; they are 1/40 mm. or 25 microns square and are never used for any purpose, except possibly in connection with the counting of bacteria in a vaccine. It will be observed that it requires four of these very small squares to make one of the squares usually designated as the small square.

There are 400 small squares in each large square, consequently, as there are nine large squares, the entire ruled surface consists of 3600 small squares. There are 4000 small squares in one cubic millimeter.

The unit in estimating the leukocyte or red cell content of blood is the cubic millimeter. The unit is 1/1000 of a cubic centimeter.

In making a leukocyte count we usually take the white pipette, which has the mark 11 just above the bulb, and draw up the blood to 0.5 and then with suction we fill the pipette to the 11 mark with the diluting fluid for which a 1/2% solution of glacial acetic acid in water is most satisfactory. This gives a dilution of 1–20.

Counting with the 2/3 inch objective all of the highly refractile dots representing leukocytes in one of the 1 mm. squares at either of the four corners we note the number and mentally multiply by 20 (the number of times the blood was diluted). As the depth of the diluted blood between the ruled surface of the hæmacytometer slide and the under surface of the cover-glass is only 1/10 of a millimeter, we multiply the figure as above obtained by 10 to get the number of cells in a 1–20 dilution of blood in a space of one cubic millimeter.

Example: Counted 90 leukocytes; $90 \times 20 = 1800 \times 10 = 18{,}000$; equals number of leukocytes in one cubic millimeter of blood.

For red counts we use the red count pipette which has the 101 mark just above the bulb. Taking up blood to 0.5 we draw up the diluting fluid to 101. This gives a dilution of 1–200. Counting the red cells in five of the aggregations of 16 small squares (1/20 mm.) thus having counted 80 small squares we have counted 1/50 of the total number of small squares in a cubic millimeter, there being 4000 small squares in a cubic millimeter. Consequently the number of red cells in 80 small squares multiplied by 50 and then by the dilution of 200 gives the number of red cells in one cubic millimeter of the blood examined.

It is well to make a second preparation and record the average of the two counts.

PRACTICAL BACTERIOLOGY, BLOOD WORK AND ANIMAL PARASITOLOGY

STITT

BY THE SAME AUTHOR

The Diagnostics and Treatment
OF
Tropical Diseases

SECOND EDITION

117 ILLUSTRATIONS 12MO 534 PAGES
CLOTH, $2.00, POSTPAID

"We can thoroughly recommend Dr. Stitt's work to every student and practitioner of tropical medicine. It contains a large amount of sound and up-to-date information presented in a concise way in a comparatively small space." (*From the Lancet, London.*)

"There are now so many books on tropical medicine that any fresh addition to the ranks will require to possess considerable merits to take a place in the front rank, but this little work of Stitt's will, we think, attain this position. Considering the space at the author's disposal it is wonderfully full. Most of the subjects are well and accurately treated, and are, in addition, well illustrated." (*From the British Medical Journal.*)

P. BLAKISTON'S SON & CO.
PHILADELPHIA

PRACTICAL

Bacteriology, Blood Work

AND

Animal Parasitology

INCLUDING

Bacteriological Keys, Zoological Tables and Explanatory Clinical Notes

BY

E. R. STITT, A. B., Ph. G., M. D.

MEDICAL DIRECTOR, U. S. NAVY; COMMANDING OFFICER AND HEAD OF DEPARTMENT OF PREVENTIVE MEDICINE, U. S. NAVAL MEDICAL SCHOOL; GRADUATE, LONDON SCHOOL OF TROPICAL MEDICINE; PROFESSOR OF TROPICAL MEDICINE, GEORGETOWN UNIVERSITY; PROFESSOR OF TROPICAL MEDICINE, GEORGE WASHINGTON UNIVERSITY; LECTURER IN TROPICAL MEDICINE, JEFFERSON MEDICAL COLLEGE; MEMBER, NATIONAL BOARD OF MEDICAL EXAMINERS; MEMBER, ADVISORY BOARD, HYGIENIC LABORATORY; FORMERLY ASSOCIATE PROFESSOR OF MEDICAL ZOOLOGY, UNIVERSITY OF THE PHILIPPINES

Fifth Edition, Revised and Enlarged

With 1 Plates and 144 Other Illustrations Containing 598 Figures

PHILADELPHIA

P. BLAKISTON'S SON & CO.

1012 WALNUT STREET

HARVARD UNIVERSITY
SCHOOL OF MEDICINE AND PUBLIC HEALTH
LIBRARY

Gift of Nu Sigma Nu Fraternity
October, 1928.
A 9.A.1918.2-A

First Edition, Copyright, 1909, by P. Blakiston's Son & Co.
Second Edition, Copyright, 1910, by P. Blakiston's Son & Co.
Third Edition, Copyright, 1913, by P. Blakiston's Son & Co.
Fourth Edition, Copyright, 1916, by P. Blakiston's Son & Co.
Fifth Edition, Copyright, 1918, by P. Blakiston's Son & Co.

THE MAPLE PRESS YORK PA

PREFACE TO THE FIFTH EDITION

Although the fourth edition of this laboratory manual of internal medicine was issued less than two years ago it has been found necessary to revise it very extensively in view of the important advances made as a result of the medical work of the present war.

In this connection much new material has been added to the section on agglutination as applied to grouping meningococci and pneumococci. The Dreyer method for interpretation of agglutination reactions of typhoid and paratyphoid infections has also been given in the section on "Immunity."

A new chapter dealing with the medical importance of poisonous arthropods and fishes has been inserted.

In the section on "Disinfectants and Insecticides" much new material has been added in particular a practical method of preparing Dakin's solution and recent investigations as to delousing methods. In view of its practical value a brief presentation of Mosenthal's nephritic test diet method of studying renal functioning will be found under "Urine." A table giving data as to the most important communicable diseases, based upon the report of a Committee of the American Public Health Association, will be found in the front linings.

A large number of the old illustrations have been replaced by new and better ones. In particular the four plates of the malarial parasites contained in former editions have been replaced by a single and more instructive one.

Notwithstanding the utmost effort at condensation it has been found necessary to increase the size of the present edition by sixty-two pages.

Douglas Boyd, Jr.
Harvard Medical School.
Boston, Mass

430 W. Taylor St.
Griffin, Georgia.

PREFACE TO THE FOURTH EDITION

As noted in previous prefaces, this manual represents the notes of a course along the laboratory side of internal medicine which has seemed to the author practical and concise.

The exhaustion of a large edition of this laboratory guide in a little more than two years would indicate a demand for a book of this character. By combining notes on points of clinical importance with laboratory details such a presentation of the subject would appear of more value to the student of internal medicine than the bare laboratory technic.

In view of the enormous advances in internal medicine since the appearance of the last edition it has been very difficult to incorporate new material and at the same time retain the pocket manual feature. This has been done, however, by the greater use of paragraphs of smaller type separating those printed in larger type, a plan which adds to the accessibility of the contents as well as lessening the number of pages. Notwithstanding such utilization of space it has been necessary to increase the contents of the book by almost one hundred pages.

To further facilitate quick reference numerous additional bold type headings of paragraphs have been introduced.

It has been a difficult matter to write concise discussions of such subjects as acidosis, anaphylaxis, deficiencies of renal functioning, etc., where space was so limited.

Practically every chapter in the book has been carefully revised and new material added to each. In some of the sections the changes in our views during the past two or three years have been so marked that it has been necessary to entirely rewrite large portions of such chapters.

A new chapter, dealing with diseases of doubtful or only recently determined etiology has been added to Part IV. In this will be found discussions of the vitamine theory in beriberi and pellagra as well as recent findings in connection with such diseases as typhus fever, Oroya fever, verruga, rat bite fever, spotted fever of the Rocky Mountains and sprue.

In the appendix a new section has been added on the chemical blood examinations, and in that on insecticides the recent views as to the best methods of destroying lice to control the spread of typhus fever and relapsing fever have been incorporated.

There has also been added to the appendix a section dealing with anatomical and physiological normals to furnish ready reference for work in the pathological or chemical laboratory.

Among the tests more recently accepted as of practical value and incorporated in this edition may be mentioned the following: Schick test for diphtheria immunity, tests for recognition of acidosis, tests for efficiency in renal functioning, Petroff's method for culturing tubercle bacilli, Wolff and Junghans' test for gastric carcinoma, Bronfenbrenner's modification of Aberhalden's technic, tests as applied to the duodenal fluid, Lange's colloidal gold test for general paresis, Fontana's spirochete staining technic, Gluzinski's gastric carcinoma test, and many others.

There have also been many new illustrations of animal parasites substituted for those in the third edition which did not appear to have sufficient teaching value.

E. R. S.

PREFACE TO THE FIRST EDITION

WHILE a member of the Naval Examining Board and examiner in bacteriology and clinical microscopy, I have during the past six years had an opportunity to judge of the qualifications of several hundred graduates of the various medical schools of the country from the standpoint of practical application in the laboratory of that which they had learned as undergraduates.

More particularly I have made it a point to ascertain from the successful candidates, while under instruction at the Naval Medical School, the features of their laboratory courses, which had seemed to them most practical; such methods being subsequently tested in our own class work.

As a result I have endeavored to incorporate in this manual methods which have been submitted to the criticism of postgraduate students from all the leading medical schools of the country, and which have been considered by them adapted to the requirements of practical, speedy, and satisfactory clinical laboratory diagnosis.

For the laboratory worker the most valuable asset is common sense and he must be able to bring to mind the possibilities of the production of various artefacts and results from trivial errors in technic. It has been my object to point out where such mistakes may arise, the reasons for obtaining results differing from those ordinarily obtained and the means employed to eliminate as far as possible such results.

We are too apt to neglect the trivial details of stains, reaction of media, and the like, yet it is only when every detail of technic has been rigidly carried out that we are in a position to judge of the significance of an object observed in a microscopical preparation.

In bacteriology, candidates were frequently able to give the cultural and morphological characteristics of all the important pathogenic organisms, yet when it was required of them to outline the procedure by which they would differentiate members of the typhoid-colon groups when encountered in a plate made from fæces, the problem appeared to them impossible. They possessed the information, but did not know how to apply it.

In practical work, organisms can only be separated culturally by the use of Keys and for this reason Keys are given at the beginning of each division of bacteria. These enable one to quickly place the organism isolated in its respective group. Only methods of differentiation which are applicable in a physician's private laboratory are given. Practical methods for making the final identification by agglutination or other immunity tests are described. Technic for immunizing animals to furnish such sera is given in detail.

The giving of the cultural characteristics in a systematic tabulated Key gives space in the notes for presenting the salient points in the pathological and epidemiological aspects of each organism.

I have endeavored to give a scientific yet practical classification of the important pathogenic moulds, a subject about which there exists greater confusion in the minds of students than for any other. In the nomenclature I have followed Gedoelst's "Les Champignons Parasites."

In the chapter on Media Making, it is believed that anyone after reading this section and following the instructions will be able to satisfactorily and without the adjuncts of a large laboratory make any kind of media. The directions as to titrations are given in detail because it is beginning to be recognized that reaction of media in bacteriology is of as great importance as staining is in blood work.

The section on Blood Work is practical and gives a method for making a Romanowsky stain which is quick and reliable. The chapter on Normal and Pathological Blood gives in a few pages the more important points to be borne in mind in considering a possible diagnosis.

While there is no difference between the laboratory requirements of medical work in the tropics and that in temperate climates, unless by reason of such measures of diagnosis being indispensable in the tropics, it has, however, been my endeavor to treat every tropical question, whether in blood work, bacteriology, or animal parasitology, in a more complete way than is usual in manuals of this character. Therefore it is believed that this little book will be of great service to the laboratory worker in the tropics.

It is only from working under Doctor Charles W. Stiles in his course of laboratory instruction in Animal Parasitology in the United States Naval Medical School that I feel justified in presenting a concise outline of the subjects in medical zoology which appear to me to be most important for the physician.

The system of arranging tables, showing the families, genera, etc.,

in which each species belongs will, it is believed, greatly simplify the matter of classification for the medical student. The points given under each parasite are believed to be practical ones. When a parasite has only been reported for man two or three times, very little space is given to it.

Part IV summarizes the various infections which may be found in different organs or excretions of the body and embraces both bacterial and animal parasites. Practical methods for examining material are also given.

The chapter on Immunity, in which the theoretical side is immediately illustrated by the practical application will tend to simplify this bug-bear of the medical student.

The illustrations have been selected with a view to bringing out points which are difficult to state briefly in the text, and furthermore they have been grouped together so that comparison of similar parasites is possible without turning from page to page.

I have in particular to thank Hospital Steward Ebeling of the Navy for his care in bringing out such details.

By reason of the authority of Braun, it has been considered sufficient to give in the tables only the proper zoological name of the parasite as given in the 1908 German edition. The synonyms have been omitted for consideration of space.

The works chiefly consulted in addition to that of Braun have been: Albutt's System of Medicine; Osler's System of Medicine; Muir and Ritchie's Bacteriology; Mense's Tropenkrankheiten; Blanchard's Les Moustiques; Guiart and Grimbert's Diagnostic; Ehrlich's Studies in Immunity; Stephens and Christopher's Practical Study of Malaria; Daniel's Laboratory Studies in Tropical Medicine; Manson's Tropical Diseases; Gedoelst's Les Champignons Parasites; Neveu-Lemaire Parasitologie Humaine; Chester's Determinative Bacteriology; Lehmann and Neumann's Bacteriology.

E. R. S.

CONTENTS

PART I. BACTERIOLOGY

PART IV. CLINICAL BACTERIOLOGY AND ANIMAL PARASITOLOGY OF THE VARIOUS BODY FLUIDS AND ORGANS

APPENDIX

BACTERIOLOGY, BLOOD-WORK AND ANIMAL PARASITOLOGY

CHAPTER I

APPARATUS

The Microscope

The most important piece of apparatus for the laboratory worker is the microscope. Very satisfactory microscopes can be purchased in this country. It is impossible to do good microscopical work unless the microscope gives and continues to give good definition and the working parts remain firm.

Folding microscope stands are now made which are perfectly satisfactory, such instruments, however, have only the advantage of occupying less space in a case so that unless the question of compactness is involved, as in an outfit for the military services or for a microscopist who travels about a great deal, the ordinary rigid horseshoe base is to be preferred.

A mechanical stage is almost a necessity in connection with blood-work and its use is advantageous in bacterial preparations. For the study of tissue sections the moving of the slide with the fingers is preferable. Therefore, the mechanical stage should be capable of ready attachment or removal. For the examination of colonies growing in Petri dishes we also use the stage unencumbered with the mechanical stage. A triple or quadruple nose-piece, according to the number of objectives used, is also indispensable.

One should always use a magnifying glass or better a lens in a tripod or an aplanatic triplet in the study of microscopic objects, prior to examination with the microscope. A dissecting microscope is better for this purpose and is very useful in dissecting mosquitoes, etc. In fact the dissecting microscope is almost essential in the examination of helminthological and entomological specimens. Of particular value is a stage forceps for orienting insects, especially mosquitoes, when examined on the stage of either a compound or simple microscope.

The following precautions should be observed to prevent injury to the microscope:

1. If the fine adjustment works through the arm of the microscope, always grasp the instrument by the pillar which supports the stage. In those microscopes, however, which are not constructed in this way the arm is made to serve in lifting the instrument.

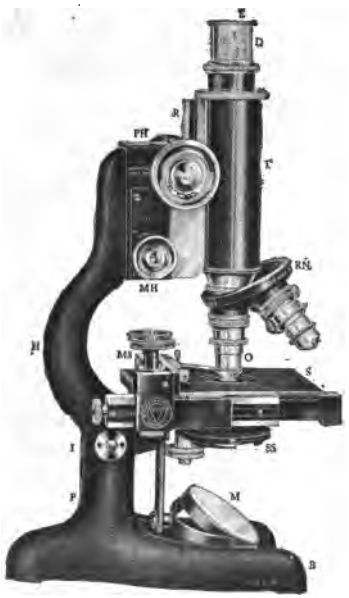


FIG. 1.—Parts of microscope. *E*, Eyepiece. *D*, Draw tube. *R*, Rack. *P.H*, Pinion head. *T*, Body tube. *M.H*, Micrometer head. *R.N*, Revolving nose-piece. *O*, Objectives. *M.S*, Mechanical stage. *S*, Stage. *H*, Handle. *S.S*, Sub-stage. *I*, Inclination joint. *P*, Pillar. *M*, Mirror. *B*, Base.

2. Always keep the microscope in its case or covered with a bell jar when not in use in order to keep away the dust. A piece of black cloth supported on a wire lamp-shade frame makes a most convenient protecting covering.

3. Alcohol ruins the lacquer of an instrument and care should be observed to keep all parts of the microscope from coming in contact with acids, alkalies, chloroform or xylol as well as alcohol.

4. Always use Japanese lens paper in wiping off the dust from dry objectives or the immersion oil from the 2-mm. one. Should one neglect to wipe off the oil from the oil-immersion objective, the dried oil can be removed by wiping with a drop of xylol on lens paper, but the cleaning should be done as rapidly as possible, with a final wiping off with dry lens paper, to avoid damage by the xylol to the setting of the lenses. Throw lens paper away after using it once.

5. Lenses are very liable to deteriorate in the tropics. One should be careful to protect his instrument from the direct light of the tropical sun.

6. If any oil is used on the mechanical parts for lubrication, all excess should be wiped off to avoid the catching of dust or gritty particles.

Objectives.—To meet the demands of clinical microscopy there should be three objectives, preferably a 16-mm. (⅔-inch), a 4-mm. (⅙-inch) and a 2-mm. (1/12-inch) homogeneous oil immersion one.

The Zeiss AA is a 17-mm. objective, and the Leitz No. 3, an 18-mm. or ¾-inch one. The Zeiss D is about 4.2-mm. and the Leitz No. 6, 4.4-mm. or ⅙-inch. A dustproof quadruple nose-piece with four objectives will be found a great conven-

ience (in addition to the ⅔-inch and 1/12-inch objectives, a ¼-inch for urine and blood counting, with a ⅛-inch for examining hanging-drop preparations and for quick examination of blood smears). An apochromatic objective costs about three times as much as an achromatic one and, except in photographic work, has little if any advantage.

Objectives are usually designated by their equivalent focal distance. It is important to remember that the equivalent focal distance does not represent the working distance of an objective, by which is meant the distance from the upper surface of the cover-glass to the lower surface of the objective. Thus a ¼-inch objective may have to be approached to the object so that the distance intervening may be only ⅙ inch or even less. This explains the frequent inability to focus an object when a high-power dry objective (⅙-inch or ⅛-inch) is used with a rather thick cover-glass—the objective possibly having a short working distance, so that the thickness of the cover-glass does not allow of any free working distance.

Oil Immersion.—In using the oil-immersion objective always dip the lens in the oil and practically touch the cover-glass—the eye being at a level with the stage—before beginning to focus. With the coarse adjustment one can feel the contact with the cover-glass, which is impossible with the fine adjustment. It saves time and disappointment to make a preliminary examination of a preparation requiring the high dry or immersion lens with a low power (⅔-inch) before employing the higher power; in this way we locate or center a suitable field for study.

It will be observed that objectives frequently have their numerical aperture marked on them. This is expressed by the letters N.A. From a practical standpoint this gives the relative proportion of the rays which proceeding from an object can enter the lens of the objective and form the image. Of course, the greater the number of rays, the greater the N.A., the better the definition, and consequently the better the objective. Immersion oil, having the same index of refraction (1.52) as glass, would not deflect rays coming from the object and so prevent their entering the objective, as would be the case if we used a dry objective with an intervening air space. In this case a portion of the rays would be turned aside by the difference in the refractive index of air. As a rule, the higher the numerical aperture, the better the objective and the less the working distance. In blood counting, the cover-glass being comparatively thick, it may happen that with a ⅙-inch of high numerical aperture there may not be sufficient working distance to bring the blood-cells into focus, which could be done with an objective of lower numerical aperture. Consequently, we must always consider the matter of working distance as well as that of numerical aperture. The skill of the optician, however, can obviate this defect in an objective of high numerical aperture so that it may combine the qualities of perfect definition with sufficient working distance.

Oculars.—As regards oculars (eye-pieces) a Leitz No. 1 and a No. 4 will best meet the requirements. For high magnification a No. 8 may

be of service. The Zeiss oculars are numbered according to the amount they increase the magnification given by the objective; thus a No. 2 increases the magnification, given by the objective alone, twice; a No. 8, eight times.

Some oculars are classified according to their equivalent focal distance and are referred to as ½-inch, 1-inch and 2-inch oculars.

A 1-inch or 25-mm. ocular magnifies the magnification produced by the objective about 10 times while a 2-inch or 50-mm. one increases the magnification of the objective four times.

A Leitz No. 0 is a 50-mm. ocular and magnifies four times. The Nos. 1, 2, 3, 4 and 5 are 40, 35, 25, and 20 mm. respectively and give eye-piece magnifications of 5, 6, 8, 10 and 12 times.

The oculars in common use are known as negative or Huyghenian oculars, by which is meant an ocular in which the lower lens (collective) assists in forming the real inverted image which is focused at the level of the diaphragm within the ocular. When using a disc micrometer, it is supported by this diaphragm, and the outlines of the image are cut by the rulings on the glass disc, and so we are enabled to measure the size of the object examined. The measurement of various bacteria, blood-cells, and parasites is exceedingly simple and assists greatly in the study of bacteria, and is indispensable in work in animal parasitology. (For details of micrometry see section on blood-work.) When an ocular is termed positive, it refers to an ocular which acts as a simple microscope in magnifying the image, the image being formed entirely by the objective and located below the ocular. By fixing one end of a hair on the rim of the diaphragm inside the ocular with a minute drop of balsam one has a satisfactory pointer to locate any particular cell in the microscopic field.

Tube Length.—The tube length is measured from the eye lens of the ocular to the end of the tube into which the objective is screwed. If a triple nosepiece is used the tube length must be shortened that much. As a rule objectives are corrected to use with a tube length of 160 mm.

Instrument makers generally specify the thickness of cover-glass to be used with a certain tube length, but as a practical matter it will be found convenient to use No. 1 (very thin) cover-glasses. The principal objection to these is that they are more fragile than the No. 2, but with a little practice in cleaning cover-glasses this is negligible. Immersion lenses are less affected than dry lenses by the question of a certain thickness of cover-glasses for a certain tube length.

One of the most fruitful causes of the crushing of microscopical objects and the overlying cover-glass or, what is far more important, the breaking of the cover-glass of a hanging-drop preparation and consequent risk of infection is the attempt to focus with the fine adjustment.

Focussing.—It should be an invariable rule for the worker to bring his objective practically into contact with the upper surface of the cover-glass, then using the coarse adjustment (rack and pinion) to slowly elevate the objective, looking through the eye-piece at the same time. In other words, obtain focus with the coarse adjustment and maintain it with the fine adjustment (micrometer screw). The fine adjustment should only be used after the focus is obtained.

Practical Points in the Use of the Microscope.—An important matter in the use of the microscope is to get all the details possible with a low power before using a higher power. This, of course, does not apply to a bacterial preparation where it is necessary to use a $\frac{1}{12}$-inch or a high-power dry lens.

It is well, however, in a bacterial or blood preparation to first examine the smear with the $\frac{2}{3}$-inch objective in order to determine suitable areas for examination with the oil-immersion objective. With tissue sections it is not only advisable to begin the study with the lowest power, but even an examination with the unaided eye or with a magnifying glass, before using the microscope, will give a surprising amount of information.

Position.—While some workers prefer to use the microscope with the body tube inclined by the inclination joint yet one gets just as good results by keeping the tube perpendicular and it is better to accustom one's self to such a position, because it is necessitated when we work with fluid mounts.

Care of the Oil-immersion.—After using the oil-immersion objective the lens should be wiped clean of oil with a strip of Japanese lens paper or with a silk handkerchief. If the oil should dry on the surface of the lens it may be removed with a drop of xylol on a piece of lens paper. Immediately afterward the lens should be dried. Dried oil on a lens often causes the lens to be considered defective. Accidental contact of the dry objectives with oil is not uncommon and should always be thought of when satisfactory optical effects are not obtainable. In depositing the drop of immersion oil on the slide bubbles are at times formed which make it almost impossible to use the $\frac{1}{12}$-inch objective. Under such circumstances I either prick the bubbles or wipe off the oil and deposit a drop anew.

It is advisable to cultivate the use of both eyes in doing microscopical work. When using one eye the other should be kept open with accommodation relaxed. It is this squinting of the unemployed eye which so often fatigues. A strip of cardboard 4 or 5 inches long, with an opening to fit over the tube of the microscope, leaving the other end to block the vision of the unused eye, will prevent the strain. This apparatus can be purchased in vulcanite.

Warm Stages.—A warm stage for the study of living protozoa may be extemporized by taking a piece of copper about the size of the stage and with a strip projecting out anteriorly for 5 or 6 inches. The under surface of the plate is covered with flannel and a hole about 1 inch in diameter cut out of the center. The proper amount of heat is applied by a flame impinging on the tongue-like projection of the copper plate.

At present there are electrically heated warm stages, connected with the desk socket by a wire and plug, which are most convenient. In fact they are almost as satisfactory as the more expensive and less convenient warm chamber or microscope oven surrounding the microscope.

Illumination.—Direct sunlight or excessively bright light is to be avoided. If such conditions must exist a white shade or muslin curtain drawn across the window is a necessity. Light from the north and from a white cloud is the most desirable. South of the equator a southern light. In the tropics a piece of plate glass fitted into the lower part of a wire screen frame gives good lighting, keeps out dust, and does not interfere greatly with the circulation of the air.

The technic in connection with proper illumination is probably more important than any other point; unless the light is utilized to the best advantage, the best results cannot be obtained. In examining fresh blood preparations or hanging drops the concave mirror should be used and the light almost shut off by the iris diaphragm so as to give a contour picture. In examining a stained blood or bacterial preparation, the Abbé condenser should be properly focused so as to best illuminate the stained film. In many instruments set-screws are provided which check the elevation of the Abbé condenser when the proper focus is reached. Inasmuch as the light from the condenser should come to a focus exactly level with the object studied, it is evident that a fixed position for the condenser would not answer when slides of different thickness were used. It is best to use a slide about 1.5 mm. thick. Always use the plane mirror when examining stained bacterial or blood films, as a color image is desired. If artificial light is used the concave mirror gives better results as such rays are not parallel. By using a 6-inch globe filled with a very dilute solution of copper sulphate and a few drops of ammonia the rays are rendered parallel and then the plane mirror should be used. Ordinarily in examining tissue sections, the Abbé condenser should either be put out of focus by racking down or by the use of the concave mirror and the narrowing of the aperture of the iris diaphragm. Swing-out condensers are now made which are very convenient. The proper employment of illumination only comes with experience, and one should continue to manipulate his mirrors, diaphragm, and condenser until the best result is obtained. Then study the specimen.

For microscopical work in a laboratory not properly supplied with windows or for night work the frosted incandescent bulb is very satisfactory.

An objection to artificial light is that one working almost entirely with sunlight forms standards and when using a different light is somewhat confused in interpretation of the microscopical picture.

There is now on the market a special electric lamp and light filter which tends to give optical effects similar to that obtained with sunlight. These are called "daylite" filters. The bulb of the lamp is filled with nitrogen gas and the glass filter

screen has a bluish color. By using a 6-inch round-bottom flask filled with an alkaline solution of copper sulphate as a condenser, similar illuminating effect to that of the "daylite" lamp can be obtained.

Dark-ground Illumination.—Very valuable information, especially as regards the detection of treponemata in material from hard chancres or mucous patches, may be obtained by the use of dark-ground illumination. There are many different types of apparatus for this purpose.

The bacteria or spirochætes are intensely illuminated and show as brilliant silvery objects in contrast to the dark background.

When the morphological details of a brightly illuminated object in the dark field can be distinctly observed it is proper to use the term dark-ground illumination. When only particles, usually surrounded by bright and dark rings, and not showing any structure, are observed in the dark field the proper designation is ultramicroscopic. In using the $\frac{1}{12}$-inch objective with dark-ground illumination a funnel-like base is supplied on which we screw the nickel-plated front mount of the objective. Before using the dark-field apparatus it must be centered with a low power. This is carried out by getting concentric rings parallel with the circle of the microscopic field. Immersion contact between the front surface of the Abbé condenser and the under surface of the slide carrying the preparation must be made before focussing the $\frac{1}{12}$th objective. As a source of illumination we may use a small arc-lamp or a Nernst lamp or an incandescent gas lamp. The light from these artificial sources should be made parallel by means of a water-bottle condenser and these rays projected on the plane mirror of the microscope. In using an arc-lamp one must have a suitable rheostat according to the electrical current employed. Information as to voltage and nature of current must be given the one supplying the apparatus.

In making preparations the slides and cover-slips should be scrupulously clean and the material thinly spread out and free of bubbles. The slides should range in thickness from 1.45 to 1.55 mm., as thicker or thinner slides fail to allow proper focussing of the illuminator on the object. With low power objectives one can obtain satisfactory dark-field illumination by pasting a circle of black paper in the center of one of the glass discs which fit in the ring under the lens of the substage condenser. The diameter of the opaque center will have to be greater as the magnifying power of the objective increases and for oil-immersion objectives a special apparatus is required. Flagellates in fæces are best studied with the dark-field illumination.

Apparatus for Sterilization

For the purpose of sterilizing glassware, media, and old cultures there are three methods ordinarily employed, the hot-air sterilizer, the Arnold sterilizer and the autoclave.

Hot-air Sterilizer.—The hot-air sterilizer, in which a temperature of about 150°C. is maintained for one hour, is ordinarily used for the sterilization of Petri dishes, test-tubes, pipettes, etc.

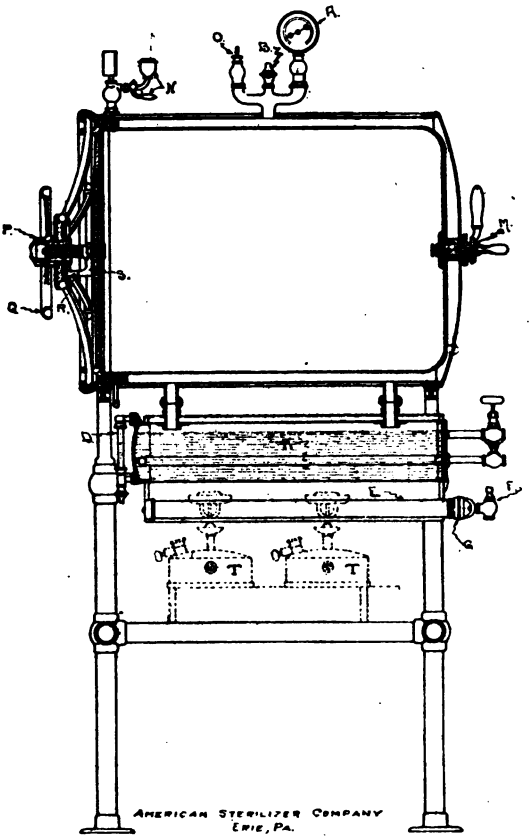


FIG. 2.—Dressing sterilizer showing cylinder containing water (K) heated either by gas or Primus kerosene lamps.

If the temperature is allowed to go too high, there is danger of charring the cotton plugs and also of causing the development of an empyreumatic oil which makes the plugs unsightly and causes them to stick to the glass. Again we must be careful not to open the door until the temperature has fallen to 60°C., otherwise there is danger of cracking the glassware. Where gas is not obtainable, the hot-air sterilizer is not a very satisfactory apparatus.

Arnold Sterilizer.—The Arnold sterilizer is to be found everywhere and can be used on blue-flame kerosene-oil stoves as readily as with gas burners. The most convenient form, but more expensive, is the Boston Board of Health pattern. The ordinary pattern, with a telescoping outer portion, answers all purposes, however.

In the Arnold, sterilization is effected by streaming steam at 100°C. It is usual to maintain this temperature for fifteen to twenty-five minutes each day for three successive days. The success of this procedure—*fractional sterilization*—is due to the fact that many spores which were not killed at the first steaming have developed into vegetative forms within twenty-four hours, and when the steam is then applied such forms are destroyed. Experience has shown that all the spores have developed by the time of the third steaming, so that with this final application of heat we secure perfect sterilization.

The Autoclave.—It is customary to use the Arnold for sterilizing gelatin, carbohydrate and milk media, even when the autoclave is at hand, the idea being that the greater heat of the autoclave may interfere with the quality of such media. The most convenient autoclave is the horizontal type, such as is to be found everywhere for the sterilization of surgical dressings. The vertical autoclaves so much used formerly are awkward to handle and require more care.

The source of heat may be either electricity, gas, the Primus kerosene-oil lamp or steam from an adjacent boiler. More recently a method of employing kerosene, gasolene, or alcohol with a gravity system has been perfected. During the past ten years, in the laboratory of the U. S. Naval Medical School, we have been using a dressing sterilizer, made by the American Sterilizer Co., with which it has been possible to most satisfactorily carry out all kinds of sterilization, thus doing away with the use of the Arnold and the hot-air sterilizer. It is impossible to sterilize ordinary fermentation tubes in the autoclave on account of the boiling up of the media and wetting of the plugs. This is still done with the Arnold. By use of the Durham tubes—which are to be preferred, except for gas analysis—sugar media can be thus sterilized provided very slight steam pressure be used. We now even inspissate blood-serum tubes at a pressure of 103°C. in the autoclave.

Should a small bubble remain in the top of the small inverted inner tube after removal from the autoclave, one may make a mark with a grease pencil at the line of the bubble; or, if preferred, the basket of Durham tubes can be heated to boiling for ten minutes in a pan of water or in the Arnold when, after cooling, the bubble will be found to have disappeared.

Sterilizing Glassware.—Glassware will come out from such an autoclave with wrappers as dry and plugs of the test-tubes as stopper-like as could be effected in a hot-air sterilizer.

The objection which exists in the use of some autoclaves, as regards condensation on dressings or apparatus, does not exist in this type. The mechanism, by

which the inner and outer chambers are connected and disconnected, and that for vacuum production, rests in the simple turning of a lever from mark to mark. We have been able with a gas burner to obtain a pressure of 15 pounds in less than ten minutes. In sterilizing test-tubes we place them in small rectangular wire baskets, 6×5×4 inches. These baskets are to be preferred to round ones, as they pack more satisfactorily in the refrigerator used for storing media. In sterilizing flasks, test-tubes, Petri dishes, throat swabs, pipettes, etc., it has been our custom, after exposing to 20 pounds' pressure for twenty minutes, to produce a vacuum for two or three minutes; then with the steam in the outer jacket for a few minutes to thoroughly dry the articles in the disinfecting chamber. The valve to the inner chamber is then opened to break the vacuum; the door is now opened, and the articles removed in as dry a state as if they had been in the hot-air sterilizer. Articles, however, can be thoroughly dried without the use of a vacuum, simply allowing the steam to remain in the outer jacket with the steam cut off from the inner chamber.

PRESSURE AND TEMPERATURE TABLE

5 pounds' pressure,	170.7°C.,	226°F.
10 pounds' pressure,	115.5°C.,	240°F.
15 pounds' pressure,	121.6°C.,	250°F.
20 pounds' pressure,	126.6°C.,	260°F.
25 pounds' pressure,	130.5°C.,	267°F.
30 pounds' pressure,	134.4°C.,	274°F.

All such articles as **Petri dishes, pipettes, swabs,** etc., are wrapped in cheap quality filter-paper, making a fold and turning in the ends as is done in a druggist's package. Old newspapers answer well for this purpose. The sterile swab can be used for many purposes in the laboratory. They are most easily made by taking a piece of copper wire about 8 inches long, flattening one end with a stroke of a hammer, then twisting a small pledget of plain absorbent cotton around the flattened end. After wrapping, the swabs are sterilized in bunches. We not only use them for getting throat cultures, but in addition for culturing fæces, pus, or other such material. The pus is obtained with a swab, which material is then distributed in a tube of sterile bouillon or water. With the same swab the surface of an agar plate is successively stroked. This method is almost as satisfactory as the German one of using bent glass rods for this purpose. Everyone has encountered the difficulties attendant upon the bending of platinum wires and also the possibility of destroying your organisms by an insufficiently cooled wire.

CLEANING GLASSWARE

It is a routine in our laboratory for everything to go through the sterilizer at 125°C. before anything else is done. This is a safe rule when dealing with dangerous pathogenic organisms (especially tetanus and anthrax).

As soon as taken out of the sterilizer the contents are emptied, and the tube or dishes placed in a 1% solution of washing soda and boiled. This thoroughly cleans them. As the washing soda slightly raises the boiling-point and also makes the spores more penetrable, it would appear that under ordinary circumstances, it would be sufficient to place all contaminated articles in a dishpan with the soda solution, and boil for at least one hour, not using a preliminary sterilization in the autoclave. The tubes are now cleaned with a test-tube brush, thoroughly rinsed with tap water and placed in a 1% solution of hydrochloric acid for a few minutes; then rinsed thoroughly in water and placed in test-tube baskets, mouth downward, and allowed

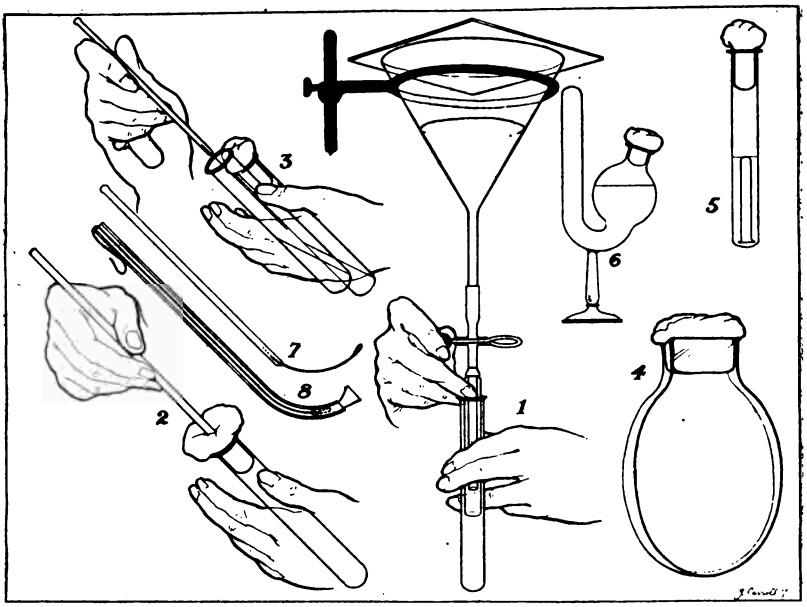


FIG. 3.—1, Filling tubes. 2, 3, Plugging tubes. 4, Culture flask plate. 5, Durham fermentation tube. 6, Smith fermentation tube. 7, Pharyngeal loop for meningococcus pharynx cultures. 8, West tube for same.

to drain over night. Some laboratory workers boil their test-tubes and other glassware in water containing soap or soap powder and, after a thorough rinsing in tap water, drain. Hydrochloric acid should not be used after the soap as it will cause the formation of an unsightly coating difficult to remove. When thoroughly dry they may be plugged and sterilized. To plug a test-tube, pick out a little pledget of plain absorbent cotton about 2 inches in diameter from a roll. Place it over the center of the tube and with a glass rod push the cotton down the tube about an inch. In culturing slow growing organisms, as tubercle bacilli, or certain pathogenic protozoa it is necessary to have plugs so prepared as to prevent drying out of the medium in the tube. The simplest way of accomplishing this is to melt some paraffin in a pan, then

removing the cotton plug with the fingers to dip the end entering the tube into the melted paraffin and then push the surface so prepared into the tube. Plasticine, sealing wax or paraffin may be used to seal over the tops of such test-tubes.

Cleaning Fluid.—The cleaning fluid commonly used in laboratories consists of 1 part each of potassium bichromate and commercial sulphuric acid with 10 parts of water. This is an excellent mixture for cleaning old slides, etc., especially when grease or balsam is to be gotten rid of. It is very corrosive, however.

In cleaning glassware for such tests as the colloidal-gold one it is essential that we use such a cleaning fluid. An efficient and less corrosive method for cleansing slides and cover-glasses is to leave them over night in an acetic acid alcohol mixture (two parts of glacial acetic acid to 100 parts of alcohol). After drying and polishing out of this mixture, it is well to pass the slides and cover-glasses through the flame of a Bunsen burner or alcohol lamp to remove every vestige of grease. Ordinarily, rubbing between the thumb and forefinger with soap and water, then drying with an old piece of linen, and finally flaming will yield a perfect surface for making a bacterial preparation.

Concave Slides, Fermentation Tubes

The concave slide is ordinarily used for making hanging-drop preparations for the examinations of bacteria as to motility, capsules, size and arrangement.

To prepare a hanging-drop preparation for the study of motility it is best to place a loopful of the young bouillon culture or a loopful of salt solution into which is then emulsified a small amount of growth from an agar slant, in the center of the cover-glass; now having applied with a brush a ring of vaseline around the concave depression in the slide we apply the slide as a cover to the cover-glass which latter adheres to the ring of vaseline. The completed hanging-drop preparation can now be turned over and placed on the stage of the microscope.

A substitute which is equally good may be made by spreading a ring or square of vaseline—smaller than the cover-glass to be used—in the middle of a plain slide. Then putting a loopful of salt solution in the center of the space, and inoculating with the culture to be studied, we finally cover it with a cover-glass, gently pressing the margins down on the vaseline. This gives a preparation for the study of motility or agglutination which does not dry out for hours, and is easier to focus upon than the concave slide hanging-drop preparation.

Fig. 4.—Hanging drop, over hollow ground slide. (*Mac Neal.*)

Hanging Drop.—In examining a hanging drop first use a low-power objective and, having brought into focus the margin of the drop as a center line, change to a $\frac{1}{6}$- or $\frac{1}{8}$-inch objective. By this procedure a thin layer of fluid is brought under the high dry objective instead of the deeper layer in the center of the drop. It is not advisable to use an immersion objective with a hanging-drop preparation.

The light should be cut down to a minimum with the iris diaphragm and the concave mirror used. When we have finished examining the preparation the cover-glass should be pushed over with the forceps so that a corner projects and we then seize this with the forceps, lift up the cover-glass and drop it into the disinfecting solution along with the slide.

FIG. 5.—Blood serum coagulating apparatus.

Fermentation Tubes.—The fermentation tube with a bulb and closed arm is expensive, difficult to clean, and is easily broken. It is, however, convenient in the determination of the gas formula of an organism. Its use is described under water analysis. As a substitute in the study of gas production and in water bacteriology, the Durham tube is to be recommended.

Into a test-tube, about 1×7 inches, we introduce the special sugar media, then drop down a small test-tube (½×3 inches) with its open end downward. Insert the plug of the large tube and sterilize. During sterilization the fluid enters the mouth of the smaller tube and fills it, and when the medium is subsequently inoculated, if gas forms, it appears in the upper part of the closed end of the smaller tube.

Inspissators.—For inspissating blood-serum slants a regular inspissator is desirable.

This is nothing more than a double-walled vessel, the space between the walls being filled with water.

FIG. 6.—Rice cooker.

As a substitute one may take the common rice cooker (double boiler). Fill the outer part with water; and in the inner compartment pack the serum tubes properly slanted on a piece of wood or a wedge-shaped layer of cotton. Place a weight on the

cover of the inner compartment to sink it into the surrounding water, and allow to boil for one or two hours. This same apparatus may be used for their sterilization on two subsequent days, but it is better to sterilize in the autoclave or Arnold. The rice cooker is of the greatest use in preparing culture media. For this purpose the outer compartment is filled with calcium chloride solution or a 25% solution of common salt, so that the temperature of the contents of the inner receptacle may be raised to the boiling point. Of course media may be prepared in an ordinary sauce-pan but there is great danger of scorching media prepared in this way. Special vessels with two bottoms, between which is an air space, are now on the market and have an advantage over the double boiler (rice cooker).

Ebony Finish.—As regards a working desk, it will be found convenient to have an arrangement similar to the ordinary flat-top desk, with a tier of drawers on each side. A block of wood with holes bored in it to contain dropping-bottles may be placed in the upper left-hand drawer. In this way the stains are as accessible as if they encumbered the desk. It is advisable to paint the inside of this drawer black so that the light may not cause the staining reagents to deteriorate.

A very popular method of preparing the wooden surfaces of laboratory desks, sinks, and tables is the application of the so-called "acid-proofing." This gives an ebony-like finish which is not affected by strong acids.

In using it the surface of the wood must be new (free of any varnish, oil, or paint, if previously so coated the surface must be planed).

Solution 1

Ferrous Sulphate	20 gm.
Copper Sulphate	20 gm.
Potassium Permanganate	40 gm.
Water, sufficient to make	500 c.c.

Apply two coats of this solution at least twelve hours between applications. When thoroughly dry apply two coats of solution No. 2.

Solution 2

Aniline oil	60 c.c.
Hydrochloric acid	90 c.c.
Water, sufficient to make	500 c.c.

When the treated surface is thoroughly dry apply one coat of raw linseed oil with a cloth. After this is dry wash with very hot soapsuds.

Useful Hints.—An aspirating bottle on a shelf elevated 2 feet, with rubber tubing and glass tip leading to a small aquarium jar or other desk receptacle, makes a good substitute for a small sink and faucet. A Hofmann screw clamp on the rubber tube controls the flow of water. The glass tube passes through a wooden clothes pin clamped on the edge of the jar.

Ordinary glass salt cellars will be found very useful, where the watch-glass is employed. They may also be wrapped, sterilized, and used to contain fluids for inoculating, etc.

A glass-topped fruit jar or a specimen jar containing a disinfecting solution for contaminated slides, etc., should be on every working desk. A good solution is that of Harrington (corrosive sublimate, 0.8; commercial HCl, 60.0 c.c.; alcohol, 400.0 c.c.; water, to 1000.0 c.c.).

Graduated nursing bottles are very convenient for stock agar. We put about 150 c.c. of medium in each bottle. These bottles are not fragile and withstand sterilization well.

There are many excellent types of water bath incubators on the market for use in Wassermann work. As a substitute in field work one may take an ordinary oval wash boiler and suspend by wire supports bent over the edges of the boiler a heavy wire gauze diaphragm. Test-tube racks containing the Wassermann tubes are supported on this diaphragm. The boiler is filled with water up to the desired height above the supporting diaphragm. For details see Fig. 141.

Disinfectant Solution.—A very simple method of making a disinfectant similar to lysol is to put one part of cresol or crude carbolic acid and one part of soft soap in a wide-mouthed bottle over night. The resulting compound (Liquor cresolis comp. U. S. P.) makes a perfect solution with water and a 5% solution of this will be found at least equal to a 5% phenol solution. In addition to using as a desk jar disinfectant it is excellent for disinfecting fæces, sputum, etc.

Platinum Loops.—For use in making loops and needles, platinum wire of 26 gauge will be found most suitable. The handle made of glass rod is preferable to the metal ones. One end is fused in the flame and, holding the 3- to 4-in. piece of platinum wire, with forceps, in the same flame, insert the glowing metal into the molten glass. By taking two lengths of platinum wire and twisting them together a more rigid needle is made for inoculating stab cultures. Loops and needles made from nichrome wire are as good as platinum ones and are very much cheaper. We saw a slit in aluminum rod handles and mash in with a vise the nichrome wire.

A platinum loop made around a piece of copper wire, 4 mm. in diameter holds about 2 mg. of culture taken up from an agar slant. Kolle estimates that an agar slant of typhoid bacilli or of staphylococci should contain 15 such loopfuls while a streptococcus slant would have almost five. It has been estimated that such a standard loop would contain between 2,000,000,000 and 3,000,000,000 organisms. Of the greatest use in culturing material obtained at autopsy is the platinum spud. This can be made by hammering out one end of a piece of 15- to 18-gauge platinum wire. In smearing out material on the surface of a plate one may use a platinum or nichrome loop. These are apt to cut the surface of the medium. Sterile applicators, with a very little cotton covering and moistened in sterile salt solution answer but the best "spreader" is a bent glass rod.

For making smears from fæces, sputum, and the like, wooden toothpicks are very convenient; the kind with the spatulate end is preferable.

Incubators.—When gas is obtainable, the maintaining of a constant temperature for the body temperature incubator (38°C.) and the paraffin oven (60°C.) is best secured by the use of some of the various types of thermo-regulators. The Reichert

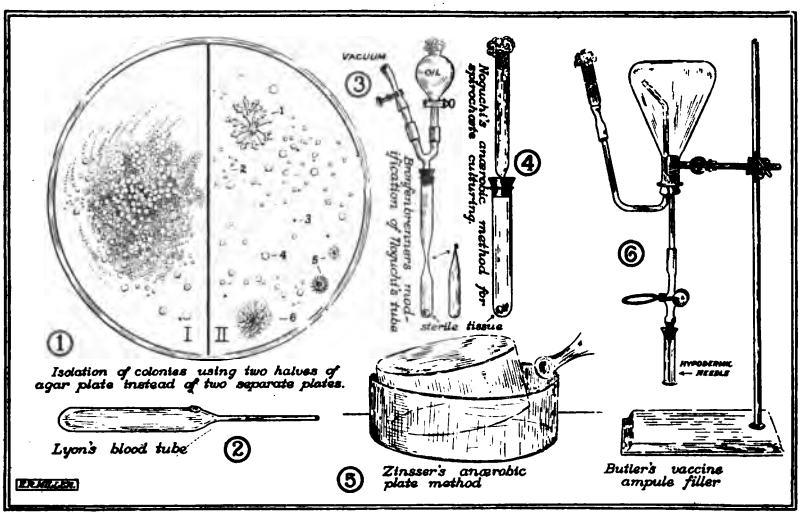


FIG. 7.—1, Method of using one plate instead of two for isolation of colonies (see page 56). The separated colonies on the No. II side of the plate are studied with unaided eye and aplanatic triplet (½ in. or ⅔ in.) both by reflected and transmitted light. After we have determined the presence of two or more different kinds of colonies, a well separated one of each type is selected and a blue pencil ring made around it on the glass surface of the back of the Petri dish together with a number. This number is carried along on culture tubes or microscopical slide preparations until the organism is identified. 2, Lyon tube for blood. More convenient than the Wright tube. For description see page 19. 3, Bronfenbrenner's anaerobic tube. This is made by drawing out a single test-tube instead of using two separate tubes as with the Noguchi method. Before drawing out the test-tube the piece of sterile tissue is introduced and after drawing out in the flame the ascitic broth or sheep serum water, as well as the material for culturing, is introduced through the drawn-out neck with a bulb capillary pipette. The lower part of the tube is placed in water at 37°C. and the vacuum connection made and after exhaustion of air the sterile paraffin oil is run in. The drawn-out portion is then sealed off in a small flame and looks like a sealed vaccine ampule. 4, Noguchi apparatus. 5, Zinsser method for anaerobic plates (page 89). 6, Butler's apparatus for filling vaccine ampules. The sterile vaccine is put in the sterile flask, and the stopper with air intake and needle filler separately sterilized and then introduced into neck of flask which is then inverted.

type is the one in general use, although there are many features about the Dunham and Roux regulators which are advantageous.

If the pressure of the gas-supply varies from time to time, it is essential to regulate this by the use of a gas-pressure regulator (Murrill's is a cheap and satisfactory one).

Incubators, controlled electrically, can be obtained of certain foreign makers, and are quoted in catalogues of American dealers. It is probable that the Koch petroleum lamp incubator is the most satisfactory one where gas is not obtainable. They should be of all metal construction, and not with a wood casing, on account of the danger from fire. They cost from twenty-five to fifty dollars.

An incubator may be extemporized by putting the bulb of an incandescent electric lamp in a vessel of water. The proper temperature may be obtained by increasing the amount of water or by covering the opening more or less completely with a towel. The test-tubes to be incubated can be put into a fruit jar or tin can, which receptacle is placed in the vessel heated by the lamp.

In emergencies we have used a chicken incubator for meningococcus plate work.

Emery suggests the use of a Thermos bottle as an incubator.

The vacuum bottle should be first warmed by pouring in warm water. Afterward the bottle should be three-fourths filled with water at 100°F.

Schrup suspends his cultures and thermometer in the water by threads attached to pins in the cork of the vacuum bottle. The plug should be paraffined or covered with a rubber cap. As regards the matter of a low-temperature incubator (for gelatin work), this may be met by using a small refrigerator. The ice in the upper part maintains an even cold, and by connecting up an electric lamp in the lower part of the refrigerator we can easily maintain a temperature which only varies one or two degrees during the twenty-four hours. The gelatin plates or tubes should be placed on the shelves usually provided with the refrigerator and not on the bottom.

With a 16-candle-power lamp a temperature of about 25°C. is maintained (this is too high, being about the melting-point of gelatin); with an 8-candle-power, one about 21° to 23°C.; and with a 4-candle-power, from 18° to 20°C.; the box being about 20×30×36 inches.

More recently we have used with entire satisfaction a low temperature incubator made by Hearson.

The low temperature is supplied by water from cracked ice packed in a large central chamber. A small dynamo controlled by a thermostat circulates the water around the chamber containing the gelatin cultures. It requires some time for proper adjustment, but afterward maintains a uniform temperature. Should the temperature of the room in which the incubator is installed be below 22°C. there is provided an automatically controlled heating coil which operates when the surrounding temperature is lower than 22°C.

Centrifuge.—When much serum reaction work is done, an electrically run centrifuge is an absolute necessity. It should be strongly constructed and placed on a firm base. There should be places for 8 tubes and the outer shell or guard should be so strong that in event of the breaking of a tube, while the centrifuge is revolving at high speed, there would be no danger for the operator. Water-power-driven centrifuges are less satisfactory and hand ones least so. The gyroscopic centrifuge is very satisfactory.

An electric drying oven is very useful, taking the place of a water bath. In reactions like that for blood acidosis, where the steam vapor interferes, it is almost essential.

Filter Pump.—A filter pump attached to the water faucet, preferably by screw threads, is almost indispensable for filtering cultures, etc., and for cleaning small pipettes, especially the hæmocytometer pipettes. Such a filter or vacuum pump with a vacuum gauge is more easily controlled. In washing red cells in Wassermann reactions a pipette attached to the rubber tubing of the pump facilitates the removal of the supernatant fluid.

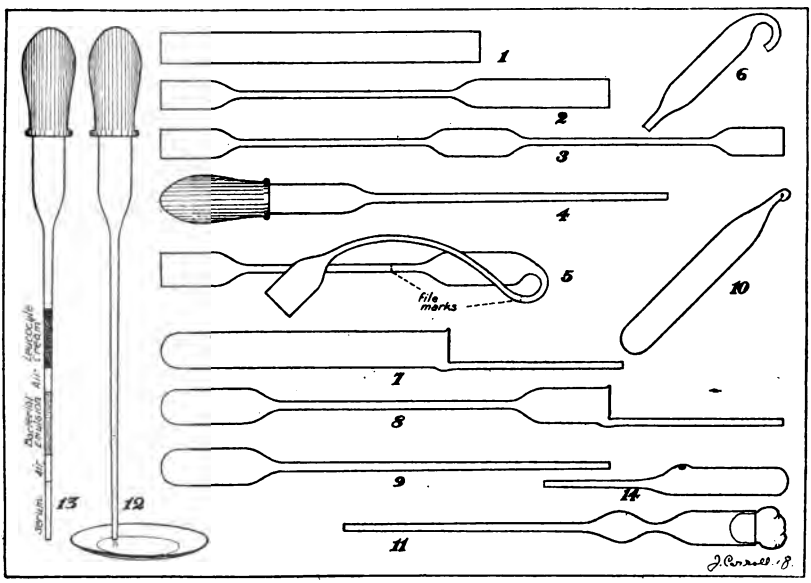


FIG. 8.—1, 2, 3, 5, 6, Wright's tube. 4, Rubber bulb pipette. 7, 8, 9, 10, Drawing out test-tubes for stock vaccine. 11, Bulb bacteriological pipette. 12, 13, Pipettes for opsonic index work. 14, Lyon's blood tube.

The filter pump is indispensable when using the various types of porcelain or Berkefeld filters. The Punkal or Muencke types of filter are the most convenient in filtering toxins or in the sterilization of certain media when heating would be unadvisable.

Capillary Pipettes.—With the possible exception of the platinum loop, there is no piece of apparatus so applicable to many uses as the capillary pipette made from a piece of glass tubing.

These may be made in a great variety of shapes. The one with a hooked end, the Wright tube, is the best apparatus for securing blood for serum tests. The crook

hangs on the centrifuge guard and by filing and breaking the thicker part of the tube the serum is accessible to a capillary rubber bulb pipette or to the tip of a hæmocytometer pipette. In this way dilutions of serum are easily made.

Lyon's Blood Tube.—Quite recently I have been using the blood tube recommended by LYON. To make it heat a 5- or 6-inch section of ¼-inch tubing in the center and draw out as for making 2 bacteriological pipettes. Divide and seal off the large end in the flame. Next seal off the capillary end. Then apply a very small flame to a

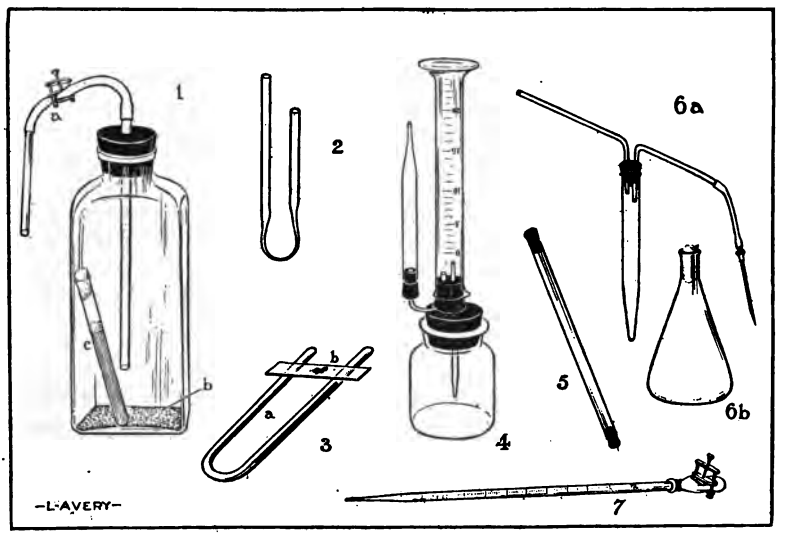


FIG. 9.—1, Apparatus combining various methods for culture of anaerobes; (*a*) Hofmann clamp for connecting with vacuum pump; (*b*) pyrogallic at bottom of bottle for Buchner's O absorption method; (*c*) deep glucose agar stab covered with sterile liquid petrolatum (see anaerobes). 2, One-fourth inch capillary loop U tube for making two nitric acid albumin tests (see chemical examination of urine). 3, Piece of tubing bent to hold slide for steaming smears in flame. 4, Schmidt's fermentation apparatus, as modified by using graduated cylinder (see under fæces). 5, One-fourth inch glass tubing, 4½ inches long with corks at each end. For centrifuging fæces for ova. 6*a*, Apparatus connected with sterile centrifuge tube for taking blood from vein of man or a guinea-pig or rabbit's heart. 6*b*, Erlenmeyer flask which can be used instead of centrifuge tube. See under sections Immunity and Blood. 7, A graduated pipette with Hofmann clamp applied to rubber bulb for precise delivery of measured quantities of liquids.

point on the large end just before it begins to taper to the capillary part. The heat causes the heated sealed off air inside to force out a blow hole. To use: Break off the sealed capillary end and allow the capillary end to suck up blood from a drop just as with the Wright tube. I consider this tube superior to the Wright one.

Pipettes.—The capillary pipette is made by taking a piece of ¼-inch soft German glass tubing, about 6 inches long, and heating in the middle in a Bunsen flame, revolving the tubing while heating it.

When it becomes soft in the center, remove from the flame and with a steady even pull separate the two ends. The capillary portion should be from 18 to 20 inches in length. When cool, file and break off this capillary portion in the middle. We then have two capillary pipettes. By using a rubber bulb, such as comes on medicine droppers, we have a means of sucking up and forcing out fluids by pressure with the thumb and forefinger of the right hand. The bulb should be pushed on about ½ to ¾ inch; this gives a firmer surface to control the pressure on the bulb.

A bacteriological pipette is made by drawing out a 9-inch piece of tubing about 3 inches at either end, then heating in the middle we draw out and have two pipettes similar to the one shown in the drawing. A piece of cotton is loosely pushed in just above the narrow portion. These may be wrapped in paper and sterilized for future use. They may be made perfectly sterile at the time of drawing out.

Illustrations are given for apparatus for culturing spirochætes as well as for anaerobic plating methods in Fig. 7. In the same cut is shown an apparatus for filling vaccine ampoules and also our aerobic plating method for class work.

Gas Substitutes.—Where gas is not at hand, the Barthel alcohol lamp gives a flame similar to that of the Bunsen lamp and is equally satisfactory for heating glass tubing. By making a collar with a lateral opening to fit the burner of a Primus lamp a powerful side-flame is obtained which is almost as suitable for glass blowing as the Bunsen blast usually employed.

The ordinary blast lamp used by plumbers is useful.

One cannot appreciate the importance of a gas supply for a laboratory until he has experienced the lack of it. At present electricity is more generally available but it cannot replace gas.

In the Philippines we had a Tirrill gasolene gas generator for 50 burners. The apparatus only costs about $300 and gives absolute satisfaction. It has a tank for the gasolene placed about 40 feet from the building. There is also a mixer and the air pump is placed in the cellar of the laboratory.

The Matthews gas machine, which weighs complete 1600 pounds, is quoted at $326.00 for a 50 burner plant.

CHAPTER II

CULTURE MEDIA

WHILE there are certain advantages in sterilizing the glass test-tubes prior to filling them with media, yet this may be dispensed with—the sterilization after the media has been tubed being sufficient. If a dressing sterilizer is at hand, this is preferable for sterilizing such media as bouillon, potato, and agar (10 to 15 pounds' pressure for fifteen minutes). Milk should be sterilized with the Arnold, subjecting the media to three steamings for twenty minutes on three successive days. Gelatin may be sterilized in either way, but preferably in the autoclave at 7 pounds' pressure for fifteen minutes. As soon as taken out of the sterilizer it should be cooled as quickly as possible in cold water. This procedure tends to prevent the lowering of the melting-point of the finished gelatin and also preserves its spissitude.

Blood-serum is preferably solidified as slants in a blood-serum inspissator. This requires one to two hours. The subsequent sterilization in the autoclave or Arnold should not be done immediately after making the solidified slants, but on the subsequent day. If done on the same day, many of the slants are ruined by being disrupted by bubbles.

Where large numbers of blood-serum tubes are to be inspissated it is more convenient to use a dressing sterilizer autoclave. A screen frame is made to fit into the inner chamber of the dressing sterilizer and set at the desired angle for proper slant. The tubes are put in and steam is turned into the outer chamber only, never permitting the heat to rise above 3 pounds or 105°C. The inspissation usually requires from forty-five to sixty minutes and the door of the sterilizer should be opened occasionally.

The preparation of blood-serum slants or slants of egg media can be conveniently carried out in a rice cooker (double boiler). Place the tubes in the inner compartment of the cooker, obtaining the slant desired by manipulating an empty test-tube, or with a towel or cotton batting on the bottom. Then cover the tubes with another towel. The outer compartment should contain water alone (not 25% salt solution). The inner compartment should be weighted down so that it is surrounded by water—

the light tubes not being sufficient to sink it. Allowing the water in the outer compartment to boil one or two hours will inspissate or solidify the slants satisfactorily. The sterilization on subsequent days may be carried out in the same apparatus, although it is more efficient if done in an Arnold or an autoclave. (This sterilization in the rice cooker makes the media too dry.)

Rice Cooker.—In making media a rice cooker is almost essential; at any rate, it is so if ease, expedition, and unfailing success in preparation are to be achieved. As it is necessary to make the contents of the inner compartment boil, the temperature of the water in the outer compartment must be raised. This is done by using a 25% solution of common salt or a 20% solution of calcium chloride in the outer compartment instead of plain water. Should $CaCl_2$ be carried over to media in inner compartment (as by thermometer) coagulation of albumin and clearing of medium will be prevented.

Makers of apparatus for the bacteriological laboratory now furnish a vessel for making media which has two bottoms with an intervening air space. This hot-air layer prevents the scorching of the media as is so liable to occur when a plain saucepan is used. Advantages over the rice cooker are that time is saved in bringing the media to a boil, and also in the maintaining of a brisk boiling temperature.

A 15% solution of salt raises the boiling point 2½°C.; a 20%, 3½°C., and a 25%, 4½°C. The raising of the boiling-point by calcium chloride is about the same for similar strength solutions.

Although the Bacteriological Committee of the A. P. H. Association recommends special steps to be taken in the preparation of gelatin and agar, yet for clinical purposes it will be found satisfactory to keep on hand a stock of bouillon, and when it is desired to make agar or gelatin to simply prepare such media from the stock bouillon in the way to be subsequently given.

Nutrient Bouillon

This may be made either from fresh meat or from meat extract. Media from fresh meat are usually lighter in color and possibly clearer. In the Philippines, however, certain measures employed for the preservation of the meat made it very difficult to prepare clear bouillon from it, so that meat extract was used entirely. There is very little difference, if any, in the nutritive power of media made in either way.

The chief objections to fresh meat as a base are: 1. It takes more time and trouble. 2. The reaction, due to sarcolactic acid and acid salts, is quite acid, so that it is necessary to titrate and neutralize the excess of acidity. 3. The reaction of the finished media tends to change unless the boiling at the time of making was very prolonged. 4. It is not infrequent to have a heavy precipitate of phosphates thrown down at the time of sterilization, thus making it necessary to repeat the process of filtration and sterilization.

If fresh meat is used, take about 500 grams (1 pound), remove fat and cut it up with a sausage mill or purchase the meat already cut up as for a Hamburg steak.

It makes little difference whether the amount be 100 grams more or less. Place the chopped-up meat in a receptacle and pour 1000 c.c. of water over it. Keep in the ice chest over night and the next morning skim off with a piece of absorbent cotton the scum of fat; then squeeze out the infusion with a strong muslin cloth, making the amount up to 1000 c.c. This meat infusion contains all the albuminous material necessary for the clarification of the bouillon. It is convenient to designate this meat base as *Meat Infusion* to distinguish from the base containing meat extract.

Having obtained 1000 c.c. of this 50% meat infusion, we dissolve in it 1% of Witte's peptone and ½% of sodium chloride. While there is a sufficiency of the various salts necessary for bacterial development in the meat juices, yet there is not enough to give the best results when bouillon cultures of various organisms are used for agglutination tests; and furthermore, when bouillon is used for blood cultures, disintegration of the red cells, with clouding of the clear medium, may occur if there be not sufficient salt present to prevent this.

The salt and the peptone are best put in a mortar, and adding about 1 ounce of the meat infusion we make a pasty mass; then we gradually add the remaining infusion until solution is complete. It is sometimes recommended to use a temperature of 50°C. to facilitate the solution of the peptone. This is not necessary, and if the temperature is not watched closely it might go up to 65°C. or higher and we should lose the clearing albuminous material from its coagulation. Of this rather cloudy solution take up 10 c.c. with a pipette and let it run out into a porcelain dish. Add 40 c.c. of distilled or rain water and about six drops of a 0.5 % phenolphthalein solution. (Phenolphthalein, 0.5; dilute alcohol, 100 c.c.) Bring the contents of the porcelain dish to a boil and continue boiling for one or two minutes in order to expel all CO_2. Now from a burette filled with decinormal sodium hydrate solution, run in this solution until we have the development of a faint but distinct pink in the boiling diluted bouillon which is not dissipated on further boiling.

It is more satisfactory to take burner from beneath the porcelain dish just before running in the N/10 solution, again boiling so soon as a pink color is obtained. Having obtained the light pink coloration we read off the number of c.c. or fractions of a c.c. of N/10 sodium hydrate solution added to produce the color. This number gives the acidity of the bouillon in percentage of N/1 acid solution.

Percent acid means that so many c.c. of N/1 acid added to 100 c.c. of the medium at the neutral point would give that percentage reaction. Thus 1½ c.c. of N/1 HCl solution added to 100 c.c. of medium at 0, would give us 1½% of acidity or +1.5. (Accurately 98½ c.c.)

Percent alkaline means so many c.c. of N/1 sodium hydrate solution added to 100 c.c. of the medium at the neutral point. Thus a ½% alkaline medium would be one whose alkalinity would correspond to the addition of ½ c.c. of N/1 NaOH to 100 c.c. of the medium at 0. It is written −0.5.

If we took 100 c.c. of the medium and put it in a beaker and then ran in N/1 NaOH solution from a burette, it will be readily understood that if we had to add 3½ c.c. of N/1 NaOH to obtain the pink color, it would show that the acidity of the 100 c.c. of medium, being tested, corresponded to 3.5 c.c. of N/1 acid solution,

and that its acidity was equal to 3½% of N/1 acid solution, or that its reaction was +3.5.

As N/1 NaOH solution is too corrosive for general use in a burette, and as 10 c.c. of medium is more convenient to work with than 100 c.c., we use a solution one-tenth the strength of the N/1 NaOH and we take only one-tenth of the 100 c.c. of medium. In this way it is the same from a standpoint of directly reading off our percentage reaction as if we had 100 c.c. of medium and used N/1 NaOH solution. The A. P. H. Association recommends 5 c.c. of the medium and the use of N/20 NaOH. As the N/10 NaOH is always at hand for titrating gastric juice, the N/10 is used instead.

Should it be found difficult to carry on the titration while boiling the end reaction may be fairly accurately determined in the cold. Deliver into a beaker from a pipette 10 c.c. of the bouillon and make up to 50 c.c. with distilled water and add 5 drops of 0.5% phenolphthalein solution. Then run in N/10 NaOH from a burette and continue to add the N/10 NaOH solution from the burette, drop by drop, until the addition of a drop fails to show any intensifying of the purplish-violet color at the spot where it came in contact with the diluted bouillon in the beaker. This marks the end reaction. A reaction of about +0.7 in the cold gives a delicate pink with phenolphthalein as an indicator. Titration in the cold is not very satisfactory with gelatin and agar.

Having determined the percentage acidity of the 10 c.c. sample tested, we easily calculate the number of c.c. of N/1 NaOH solution required to be added to the 1000 c.c. of bouillon to obtain a reaction corresponding to the neutral point of phenolphthalein. It is more exact to take the average of two titrations.

As 100 c.c. of medium would require 3½ c.c., 1000 c.c. would require 10 times as much, or 35 c.c. N/1 NaOH solution. Having measured out and added 35 c.c. of the N/1 NaOH solution to the meat infusion, containing salt and peptone, we have a solution which is exactly neutral to phenolphthalein, or 0. It is usually considered that a reaction of about 1% acid is the optimum reaction for bacterial growth. Hence we should now add 1% of N/1 HCl solution to the medium. This would be accomplished by adding 10 c.c. of N/1 HCl solution to the 1000 c.c. of neutralized medium, and we would have a medium with a reaction of +1. If we desired a reaction of 1% alkalinity we would add an additional c.c. of N/1 NaOH solution to every 100 c.c. of the medium at 0, or 10 c.c. for the 1000 c.c. of medium. The reaction would then be −1.

As a matter of convenience, we usually determine the reaction of the medium, which is always more or less acid, and then add enough N/1 NaOH to reduce the acidity to the percentage we desire to set the medium, instead of neutralizing all the acidity present and then, in a second operation, restoring the acidity to the point desired.

Thus finding the acidity of the medium to be 3½% and desiring to give it an acidity of 1%, we would add only 2½ c.c. of N/1 NaOH to every 100 c.c. of medium, or 25 c.c. for the 1000 c.c. of medium. The reaction would then be found to be + 1.

The neutral point of litmus is not a sharp one, but it corresponds rather closely with a reaction of +1.5 to phenolphthalein. The recommendations of the A. P. H. Association call for making the titration with the medium boiling. If the color of the end reaction at boiling-point be obtained, it will be found that when cool it deepens until it corresponds to the rich violet-pink of the end reaction in the cold.

Hydrogen-ion Concentration.—There is a tendency in elaborately equipped laboratories to substitute this more accurate method of standardization of reaction for the one just described. The medium is compared with standard colorimetric solutions.

A concentration of PH = 7.35, which is about that of the blood, has been found the most satisfactory hydrogen-ion concentration for media for growing delicate pathogens.

To summarize the preparation of bouillon:

Take Peptone	10 grams
Sodium chloride	5 grams
50% meat infusion	1000 c.c.

Dissolve the peptone and sodium chloride in the meat infusion and add enough N/1 NaOH to make the reaction +1.

Put the solution in the inner compartment of a rice cooker and bring to the boiling-point and maintain this temperature for twenty minutes. The calcium chloride or sodium chloride in the outer compartment of the rice cooker enables us to secure a boiling temperature for the contents of the inner compartment. Do not stir the bouillon that is being heated, as the pultaceous membranous mass of coagulated albumin makes filtration easy. Filter. The filter-paper in the funnel should be thoroughly wet with water before pouring on the bouillon. This is to prevent clogging of the pores of the filter-paper. Make up the quantity of filtrate to 1000 c.c. by adding water.

If greater exactness is demanded than answers for ordinary clinical work, it is advisable to again titrate and again adjust the reaction or to simply record the exact reaction. It is more convenient to have a counterpoise to balance the inner compartment and then to add water to the medium until a kilo weight, in addition to the weight balancing the container, is just balanced. Then titrate, adjust the reaction (if so desired), and filter. Sterilize in the autoclave at 115°C. for fifteen minutes or in the Arnold on three successive days. The use of a balance is preferable in the preparation of bouillon, necessary in making gelatin and imperative in making agar media.

Bouillon Made from Liebig's Meat Extract

Place in a mortar 3 grams of Liebig's extract, 10 grams of peptone and 5 grams of sodium chloride. Dissolve the whites of one or two eggs in 1000 c.c. of water. Then add this egg-white water, little by little, to the extract, peptone, and salt in the

mortar until a brownish solution is obtained. Pour this into the inner compartment of a rice cooker; apply heat to the outer compartment containing the salt or calcium chloride solution, allow to come to a boil and to continue boiling for fifteen to twenty minutes. Do not stir. Place inner compartment on the scales and its counterpoise and a one-kilo weight on the other side. Add water until the two arms balance. Filter and sterilize.

The reaction of media made with Liebig's meat extract rarely exceeds +0.75 (from +0.6 to +0.9). Consequently for growing bacteria it is unnecessary to titrate and adjust reactions unless precision is demanded.

Sugar-free Bouillon

Inoculate nutrient bouillon in a flask with the colon bacillus. Allow to incubate at 37°C. over night. Pour the contents into a sauce-pan and bring to a boil to kill the colon bacilli. Put about 15 grams of purified talc (Talcum purificatum, U. S. P.) in a mortar. Add the dead colon culture, stirring constantly. Then filter through filter-paper. It may be necessary to again pass the filtrate through the same filter until the sugar-free bouillon is perfectly clear.

For ordinary purposes the very small amount of sugar in bouillon made from Liebig's meat extract may be neglected in determining gas production; so that under such conditions the various sugars could be added directly to the meat-extract bouillon. Dunham's peptone solution may be used as a substitute for sugar-free bouillon, the sugars being added to it. We prefer the serum water medium of Hiss.

Sugar Bouillon

The sugar media ordinarily used for determining fermentation or gas production are those of glucose and lactose. In special work such carbohydrates as saccharose and maltose are used. The alcohol mannite is used in differentiating strains of dysentery bacilli.

To make, simply dissolve 1 or 2 %of the sugar in sugar-free bouillon or that made from meat extract. Tube in Durham's or the ordinary fermentation tubes and sterilize in the autoclave at only about 5 pounds' pressure for fifteen minutes, or in the Arnold. Ordinary peptone solution is a good substitute for sugar-free bouillon.

Too high a degree of heat may turn the sugar bouillon brownish. The nature of the sugar itself may further be affected by too high a temperature. Many of the carbohydrates added to bouillon are liable to be split up on subjection to any marked sterilization. *Maltose* is particularly unstable. It is best to make about 20% solution of the carbohydrates in distilled water and sterilize in small flasks adding enough to the sterile bouillon to give a 1% solution. *Inulin* usually contains resistant spores so that its sterilization may need the autoclave. One of the great difficulties about reporting on sugar reactions is the possibility of not working with a chemically pure sugar as well as with one changed by too much heat. Inulin is a polysaccharide resembling starch but does not give the iodine reaction. It is obtained from the roots of chicory or dandelion. For further notes on carbohydrates see water analysis. We color our sugar bouillon as noted under Andrade's reagent.

Besredka's Egg Bouillon

100 c.c. broth without salt; 80 c.c. 10% egg-white solution; 20 c.c. 10% yolk of egg solution.

The egg-white solution is prepared by constant heating while adding distilled water little by little. It is then filtered through absorbent cotton and heated to 100°C. Then filter through filter-paper.

This opalescent liquid is then put into tubes or flasks and sterilized at 115°C. for twenty minutes.

To 100 c.c. of 10% emulsion of egg yolk in water you add about 1 c.c. of N/1 NaOH solution. One should add enough of this caustic soda solution to clarify the emulsion slightly; it should still be opaque when in a rather thick layer. Heat to 100°C., then filter and finally sterilize at 115°C. for twenty minutes.

The bouillon is a 50 to 75% meat infusion with peptone.

This is an excellent medium for growing pneumococci, streptococci, meningococci and gonococci; even the organism of pertussis will grow on this medium. Of course such organisms as the typhoid-colon group, cholera, diphtheria and pathogenic anaerobes grow readily on it.

The medium is particularly recommended for growing tubercle bacilli. For this purpose the peptone in the bouillon is omitted as it is found that tubercle bacilli grow better without it. Besredka found that tubercle bacilli grew better when he omitted the addition of glycerine to his medium.

It will be noted that for tubercle bacilli the medium should contain neither peptone, salt nor glycerine.

In the course of two or three weeks we have a thick membranous growth.

It is stated that after a growth of from four to six weeks human strains show a dry scale-like growth which is easily detached from the sides of the flask, while bovine cultures show a sort of mucoid growth adhering to the walls.

Egg Broth.—This has been extensively used in the war zone for growing anaerobes. Emulsify one whole egg in 300 c.c. water. Bring slowly to a boil with frequent shaking. Then tube and sterilize. The organism of malignant œdema grows particularly well in it.

Calcium Carbonate Bouillon

Where we wish to cultivate such organisms as streptococci and pneumococci in massive cultures we may add small fragments of marble (calcium carbonate) so that any inimical excess of acid may be neutralized. North used a glucose bouillon containing calcium carbonate in the production of massive cultures of *B. bulgaricus.*

Glycerine Bouillon

Add 6% of glycerine to ordinary bouillon. It is chiefly used in the cultivation of tubercle bacilli.

Peptone Solution (Dunham's)

Dissolve 1% of Witte's peptone and ½% of sodium chloride in distilled water. Filter, tube, and sterilize. Peptone solution may be used as a base for sugar media

instead of bouillon. It is the medium used in testing for *indol production*. This test is made by adding from 6 to 8 drops of concentrated H_2SO_4 to a twenty-four- to forty-eight-hour-old peptone culture of the organism to be tested. If the organism produces both indol and a nitroso body, we obtain a violet-pink coloration, "cholera red." If no pink color is produced on the addition of the sulphuric acid, add about 1 c.c. of an exceedingly dilute solution (1 : 10,000) of sodium nitrite.

Cholera Red.—It is very important in determining the "cholera red" reaction to know that the peptone used will give the reaction as it is not given by true cholera strains with certain samples of peptone.

For the Voges-Proskauer Reaction.—Fill fermentation tubes with a 2% glucose Dunham's peptone solution and sterilize. After inoculation with the organism to be tested incubate for three days. Then add 2 to 3 c.c. of strong caustic potash solution. The development of a pink color on exposure to the air is a positive reaction (the color of a weak eosin solution).

Hiss' Serum Water Medium

Take one part of clear beef serum and add to it about three times its bulk of water. Heat the mixture in the Arnold for fifteen minutes to destroy any diastatic ferment which might be present. Color to a deep transparent blue with litmus solution and then add 1% of any of the various sugars used in fermentation tests. Sterilize in the Arnold by the fractional method.

Nutrient Agar

In making agar medium it is preferable to use powdered agar, as this goes into solution more readily than the shredded agar. The reaction of agar is slightly alkaline, so that if 1½ to 2% of agar is added to nutrient bouillon having a reaction of +1 the finished product will be found to be about +0.8.

To make: Weigh out 15 to 20 grams of powdered agar and place in a mortar. Make a paste by adding nutrient bouillon, little by little, and when a smooth even mixture is made, pour it into the inner compartment of a rice cooker and add the remainder of the 1000 c.c. of bouillon. The use of the balance is preferable.

The outer compartment of the rice cooker should contain the 25% salt solution. Bring to boil, and the agar will be found to have entirely gone into solution after five to ten minutes of boiling.

Then, using a funnel which has been heated in boiling water and which contains a small pledget of absorbent cotton, or the cotton between two layers of gauze, we filter the agar, tube it, and sterilize it in the autoclave or Arnold. One and one-half percent agar can be readily filtered through filter-paper and gives a clearer medium.

Some prefer to place the filter stand, funnel with gauze cotton filter and flask in an Arnold sterilizer, for twenty minutes, so that when taken out the funnel will not only be hot but the filter, being moist, will allow of more rapid filtration.

By taking of meat extract 3 grams, peptone 10 grams, salt 5 grams, powdered agar 15 grams, the white of one egg and 1000 c.c. of water, making at first a paste of all the ingredients in a mortar, then gradually

adding the remainder of the 1000 c.c. of water, putting in the rice cooker, bringing to a boil without stirring, allowing to boil fifteen minutes and then filtering through absorbent cotton placed between two layers of gauze in a hot funnel, we obtain a satisfactory medium, the reaction of which will be from +0.7 to +0.9. It is very important not to interfere with the pultaceous coagulum which forms on the surface of the boiling agar.

Where very exact adjustment of the reaction of the finished product is desirable the method of preparation of the Committee on Water Analysis of the American Public Health Association is to be preferred.

Dissolve 15 grams of agar in 500 c.c. of water in the inner compartment of the rice cooker previously described. After the agar is in solution (after ten to fifteen minutes boiling) remove the inner compartment, containing the 3% agar solution, and allow it to cool to about 55°C. Mix in the mortar, as described in the directions for making nutrient bouillon from Liebig's extract, 3 grams of Liebig's extract, 10 grams of peptone and 5 grams of sodium chloride in 500 c.c. of water containing the whites of one or two eggs. Heat this mixture to 50 to 55°C. and pour it into the agar solution, in the inner compartment, which has been cooled to about 55°C. Now titrate this mixture containing 500 c.c. of double strength agar and 500 c.c. of double strength peptone, meat extract and salt solution. The resulting 1000 c.c. gives 1½% agar and 1% peptone solution. Having adjusted the reaction by the addition of the necessary amount of N/1 acid or alkali, we place the inner compartment in the outer one of the rice cooker, bring to a boil and filter through filter-paper which has been wetted with boiling water. The filtration can be carried out in the autoclave or in an Arnold sterilizer. Of course the ordinary filtering through gauze and cotton will answer where clearer media is not an object.

A very convenient and satisfactory method of making an agar base in large quantities for use with special media, such as blood agar, laked blood agar and the like is the following: Make the solution of Liebig's extract, peptone, salt and agar (2 or 3%) with or without white of egg and place in a boiler of the rice cooker or any other large straight-sided granite iron receptacle. Then put in chamber of the dressing sterilizer and heat between 5 and 10 pounds (110 to 115°C.) for at least forty-five minutes. Take the solution out of the sterilizer, adjust the reaction and put back in the sterilizer for thirty minutes at the same temperature as before noted. The greater the heat or pressure applied the darker the medium will be, so that a pressure not exceeding 3 pounds would be satisfactory and give a lighter colored medium. The medium may be then filtered through cotton gauze or left in the sterilizer over night (steam being turned off). In the morning the jelly-like mass will have solidified with the sediment at the bottom. This jelly mass can be dumped out of the container and the bottom portion removed. This fairly clear jelly can then be melted in the autoclave and deposited in flasks for subsequent sterilization. We use nursing bottles for storing this agar base instead of test-tubes or flasks. It is convenient to add the blood or serum directly to the melted agar base contained in the nursing bottles.

Glucose Agar

Add the agar to 1 or 2% glucose bouillon and proceed as for ordinary agar. If preferred, the glucose agar can be made by rubbing up meat extract 3 grams, peptone 10 grams, salt 5 grams, glucose 10 grams and 15 grams of agar in 1000 c.c. of water containing the white of egg (one to two eggs), then boiling in the rice cooker and filtering.

Glycerine Agar

Add the agar to 6% glycerine bouillon instead of nutrient bouillon, or the glycerine may be added to nutrient agar which has been melted. Glycerine agar with a reaction of 0 makes an excellent base for blood and serum media for use in culturing delicate pathogens.

Glycerine Agar Egg Medium

Take the white and the yolk of one egg and mix thoroughly in a vessel kept between 45° and 55°C. with an equal amount of glycerine agar. Tube the medium, inspissate in a rice cooker as for serum tubes, and sterilize as for blood-serum tubes.

This makes an excellent medium for growing tubercle bacilli. As egg medium has a tendency to be dry, it is well to add 1 c.c. of glycerine bouillon to each slant before autoclaving.

Vedder's Starch Medium

Macerate 500 grams of beef in 1500 c.c. of water in ice box over night. In the morning filter into stew pan through doubled gauze. Bring fluid slowly to a boil and add agar 1½%. Boil for from twenty to thirty minutes.

Titrate and correct reaction to 0.2 to 0.7 acid. Check reaction to show faint blue on red litmus.

Let cool to about 60°C. and add two whole eggs, well beaten, to clarify. Bring very quickly to a boil, then turn down flame and allow to simmer until a complete coagulum forms.

Filter through gauze and cotton and add 1% starch.

Let stand in Arnold for about forty-five minutes, shaking about three times during this period, to distribute the starch.

Tube or flask, and sterilize in autoclave for fifteen minutes at 10 pounds. There must be plenty of water of condensation and the agar must not exceed 1½%.

This is especially recommended for culturing gonococci. English workers have used it in isolating meningococci from carriers. For this purpose the starch agar has 1% of glucose added to it and colored with litmus solution. The *Meningococcus* acidifies the glucose while the *M. Catarrhalis* does not.

Nutrient Gelatin

Place in a mortar 3 grams of Liebig's extract, 10 grams of peptone and 5 grams of sodium chloride. Dissolve the whites of one or two eggs in 1000 c.c. of water. Then add this egg-white water, little by little, to the meat extract, peptone and salt, in the mortar, until a brownish solution is obtained. Pour this into the inner com-

partment of the rice cooker and bring the temperature up to 45°C. (This preliminary elevation of temperature is better carried out in some heated water in a pan, as the heating by means of the salt solution in the outer compartment of the rice cooker is difficult to control, so that a temperature approximating 70°C. might be obtained and the albumin of the white of egg coagulated. The temperature in the outer compartment might be approaching boiling before the contents of the inner compartment would show 45°C.) Now take about 120 grams of "gold label" or other good quality gelatin (12%) and crush it down in the meat extract egg-water solution in the inner compartment of the rice cooker.

The gelatin quickly goes into solution at 45°C. Gelatin being quite acid it will probably be found upon titration that the reaction is about +4%. N/1 NaOH solution is added to bring the reaction to about +1% or 3 c.c. of N/1 NaOH for each 100 c.c., provided the reaction were exactly +4%. The procedure is the same as for bouillon. The color reaction is not quite as distinct with gelatin as with bouillon.

Having neutralized and allowed to boil for fifteen minutes, we filter through filter-paper in a hot funnel. As it is very important that gelatin should be perfectly clear, it is better to filter through filter-paper than through cotton. The filter-paper should be very thoroughly wetted with very hot water before filtering gelatin or agar.

Tube the medium and sterilize, either in the Arnold on three successive days or in the autoclave at 8–10 pounds' pressure for ten minutes. The tubes should be cooled as quickly as possible in cold water after taking out of the sterilizer.

Agar Gelatin Medium (North)

Lean chopped beef or veal	500 grams.
Agar	10 grams.
Gelatin, Gold label	20 grams.
Peptone, Witte's	20 grams.
Sodium chloride	5 grams.
Distilled water, *q.s.*	1000 c.c.

Extract the chopped beef with 500 c.c. distilled water for eighteen hours, strain through muslin and combine the ingredients in the usual way. Adjust the reaction to the neutral point, using phenolphthalein as indicator.

North states that this medium is excellent for streptococci, pneumococci and diphtheria bacilli because it is soft, moist, and can be used at 37°C.

It is claimed to be of special value for carrying stock cultures. It is useful as a plating medium for milk.

Litmus Milk

Milk for media should be as fresh as possible. It should then be put in a 1000-c.c. Erlenmeyer flask, sterilized for fifteen minutes in the Arnold, and set over night in the refrigerator. The next morning the milk beneath the cream should be siphoned off. The short arm of the siphon should not reach the bottom of the flask so as to avoid the sediment. Add sufficient litmus solution to this milk to give a decided lilac tinge; tube and sterilize in the Arnold on three successive days.

Litmus milk which apparently is as satisfactory as the above as regards nutritive quality and cultural characteristics can be made from certain canned milks which have not been condensed or sweetened and which do not contain chemical preservatives. The "Natura" brand of milk is the one I have experimented with.

Litmus Solution.—A simple solution may be made by digesting the powdered cubes repeatedly with hot water, mixing the extracts, and, after allowing them to stand all night, decanting the solution from the inert sediment into a clean bottle.

In litmus solution so made, however, a red dye is also present while calcium and other salts are dissolved out. For bacteriological purposes a pure solution of the blue dye should be used. This is called "azolitmin." It is freely soluble in water but insoluble in alcohol.

It can be conveniently prepared as follows: Weigh out 2 ounces of powdered litmus; digest repeatedly with fresh quantities of hot water until all the coloring matter is dissolved out; allow to settle, and decant off the fluid from the insoluble powder. Add together the extracts, which should measure about a liter. Evaporate down the solution to a moderate bulk, then add a slight excess of acetic acid, so as to convert all carbonates present into acetates. Continue the evaporation, the later stages over a water bath, until the solution becomes pasty. Add 200 c.c. of alcohol, and mix thoroughly. The alcohol precipitates the blue coloring matter, while a red coloring matter, together with the alkaline acetate present, remains in solution. Transfer to a filter. Wash out the dish with alcohol and add this to the filter. Wash the precipitate on the filter with alcohol. Dissolve the pure coloring matter remaining on the filter in warm distilled water and dilute to 500 c.c. Azolitmin solution prepared in this way is more sensitive than ordinary litmus solution.

Azolitmin in powder can be purchased from dealers in chemicals.

Andrade's Indicator

This indicator has come into general use as a substitute for the litmus indicator as showing acid production in carbohydrate media.

We have recently been using neutral red to color glucose fermentation tubes, Andrade indicator for lactose and litmus for saccharose. These sugar bouillons are tubed in 150-mm. tubes. In smaller tubes (100 mm.) we use neutral red for maltose, Andrade for mannite and litmus for inulin.

In this way we do not need to label our uninoculated media. To make Andrade's indicator take of acid fuchsin 0.5 gm. and distilled water 100 c.c. To this magenta red solution add N/1 NaOH until the color changes to pink, then to brownish red and then to yellow. Shake the reagent after each addition of the alkali. Usually it takes about 17 c.c. of normal sodium hydrate solution to decolorize 100 c.c. of the fuchsin.

Add 1 c.c. of the decolorized reagent to 100 c.c. of any sugar bouillon. The broth is colorless at room or incubator temperatures, but pink at 100°C. If an organism produces acid it turns the indicator magenta red.

Potato Slants

Take Irish potatoes and scrub thoroughly with a stiff brush. Then pare off generously all the outer portion. From the white interior cut out cylinders with a

cork borer. These cylinders should be of ½ to ¾ of an inch in diameter. Divide a cylinder by a diagonal cut. This gives a plug with a flat base, the other extremity being a slant. These potato plugs should be left in running water over night or washed with frequent changes of water. This prevents the blackening of the plug. Into a 1-inch test-tube drop a pledget of absorbent cotton well moistened with water. Then drop in the potato plug, base downward. Sterilize in the autoclave at 15 pounds for fifteen to twenty minutes, to insure sterility.

For glycerine potato, soak the plugs in 6% glycerine solution for about one hour. Then drop a pledget of absorbent cotton moistened with the same glycerine solution into the test-tubes and follow it with the potato plug. Sterilize in the autoclave.

Blood-serum

The blood of cattle should be collected in large pans or pails at the abattoir. This vessel of blood should then be kept in the cold-storage room and the next morning the more or less clear serum will have been squeezed out from the clot. Collect this serum and keep in the ice chest for future use. If to be kept for a long time, it is advisable to add about 2% of chloroform to the serum in tightly corked flasks. This will not only keep the serum, but will eventually sterilize it.

To make *Löffler's serum*, take 1 part of glucose bouillon and 3 parts of blood-serum. Mix, tube, and coagulate the albumin in the inspissator or rice cooker, giving the tubes a proper slant before heating. Sterilize the following day in the autoclave as previously directed (7 pounds) or in the Arnold on three successive days. See directions on page 21.

A Substitute for Ordinary Blood-serum

Add from 10 to 15 c.c. of 1% glucose bouillon to the white and yolk of one egg, make a smooth mixture in a mortar and tube. Inspissate and sterilize as for ordinary serum slants. The morphology of the diphtheria bacilli and the luxuriance of growth is similar to that of cultures on Löffler's serum.

When this medium is to be used for culturing tubercle bacilli add about 1 c.c. of glycerine bouillon to each tube before final sterilization in the autoclave. The cotton plugs should be paraffined to prevent drying of the slants in the incubator. This medium seems to answer as a substitute for Dorsett's egg medium. (While glycerine bouillon favors growth of human tuberculosis, it is not so satisfactory for bovine tuberculosis as plain glucose bouillon.) This is better than the various white of egg substitutes usually recommended. (Pouring a little alcohol in the mortar and moistening the sides by tilting, then burning off the alcohol, in a measure sterilizes the mortar. If the egg is cracked open with a sterile knife, a medium can be prepared which will be sterile as the result of the two-hour inspissation in the rice cooker.) By covering the tube with a rubber cap or preferably, by heating the plugged end of the test-tube, quickly withdrawing the cotton plug and dipping the part of the plug which enters the tube into hot melted paraffin, then quickly reintroducing the plug, the contents of the tube will be prevented from drying out. This procedure is essential for growing tubercle bacilli.

Egg media are excellent for culturing anaerobes. One can add about 5 drops of 1% neutral red aqueous solution to each egg, inspissating as above. The reddish color appears in colonies producing acid and is of value in anaerobic work to make such colonies more distinct.

Dorsett's Egg Medium

This is prepared by breaking whole eggs into a sterile flask, mixing thoroughly then adding 25 c.c. water to every 4 eggs, straining through a sterile cloth and tubing 10-c.c. quantities. These tubes are slanted in an inspissator and kept at 73°C. for four or five hours on two successive days. On the third day a temperature of 76°C. is applied. Before inoculating add 3 or 4 drops of sterile water to each tube. The tuberculous material should be rubbed into the surface well and the plugs paraffined.

Hydrocele, Serum, Ascitic and Milk Agar

To tubes of melted agar at 50°C. add from 1 to 3 c.c. of hydrocele or ascitic fluid, observing aseptic precautions. Allow the agar to solidify as a slant, or as a poured plate.

For milk agar we add 2 or 3 c.c. of plain or litmus milk to a tube of melted agar. This makes an excellent plating medium for *B. bulgaricus*. When poured into a plate it is opaque but the colonies stand out well.

For obtaining sterile serum we generally use the apparatus described under Blood Agar and as a rule take the blood from vein of arm in man or jugular vein of neck of sheep used for Wassermann work. The sterile serum separates from the clot and can be pipetted off with a sterile pipette and added to the melted 2 to 3% agar as above. A method recommended by Fildes is to take blood at the slaughter house in sterile vessels, allow to clot and remove serum. Five c.c. of ether is added to each 100 c.c. in a glass-stoppered bottle of which the stopper is fixed in and the mixture heated for one hour in a water-bath at 45°C. after which it is placed in the incubator at 37°C. for several days, by which time the serum is sterile. Before using the ether is driven off at 45°C. This is better than the old method of sterilizing with 2% chloroform.

Blood Agar

For obtaining blood to make blood agar we use the apparatus described under blood culturing and shown in Fig. 9. We take human blood from the arm vein, sheep blood from the neck vein, or rabbit blood from the heart. In the Erlenmeyer flask of 100 c.c. capacity is placed 5 c.c. of sterile 10% sodium citrate solution provided we want to take 50 c.c. of blood from sheep or man. The final mixture to prevent coagulation should contain about 1% sodium citrate. As a rule we only take about 25 c.c. from man or rabbit so 2½ c.c. of 10% citrate would suffice. The perforated rubber stopper is removed and replaced with the sterile cotton plug of the flask and the fluid mixture pipetted off and added to the melted agar.

Mixture is facilitated by rotating the tube rapidly between the hands. The medium may be slanted or poured into plates. As this medium is satisfactory for the growth of hæmoglobinophilic organisms, as well as for others, we use it as a routine plating medium, the others being nutrient agar and Endo.

Special Media for Meningococcus Plates

These media may be either (1) blood agar citrated or defibrinated, (2) laked blood agar, (3) Huntoon's heart muscle hormone agar, (4) agar plates to which a few drops of whole blood have been added—not sufficient blood to cause the plate to be opaque.

Briefly the preparation of each is as follows:

(1) (*a*) To 100 c.c. of agar 2% to 3% (+ 0.3 phenolphthalein) is added 20 c.c. of a mixture of equal parts of blood and sodium citrate solution (1% in normal saline). Ten plates are poured, each containing 1 c.c. of whole blood. This plate is opaque.

(*b*) To 150 c.c. of agar (same as above) add 5 c.c. of defibrinated blood (rabbit, goat, or human). Ten plates are poured, these being less opaque than (*a*).

(2) To 100 c.c. of agar (as above) 10 c.c. of laked blood are added, 10 plates being poured. The blood may be laked with ether or distilled water.

(3) To 100 c.c. tap water add 500 grams of ground beef heart. Bring to boiling point and immediately titrate. Bring the reaction to neutral to phenolphthalein and add 1 c.c. of 4% NaOH. Place in Arnold sterilizer for one hour, take out and skim. Sterilize in Arnold for one hour on three successive days, filter, add 1% glucose, and tube. In filtering use only glass wool or wire gauze; never use cotton, gauze, or filter-paper.

(4) To 100 c.c. of agar add 20 to 30 drops of a mixture of equal parts of blood and 1% sodium citrate. Pour 10 plates.

The main advantage of the whole blood plate is the detection of hæmolytic organisms whose colonies resemble those of the meningococcus. These organisms cannot be distinguished from meningococcus on laked plates until one has become *exceedingly* familiar with the characteristics of the latter colony. However, the opacity of the heavier whole blood prevents the detection of meningococcus by its appearance in transmitted light, and one has to rely on the appearance of the colony on the surface of the plate. The colonies, however, are generally larger on this richer medium than on agar containing less protein.

The transparency of the colony and its lenslike appearance show very well on laked blood and on the media containing very small amounts of whole blood. However, we have found that the colonies on weak whole blood plates are exceedingly small and are very easily overlooked when rapidity of examination is essential. Hæmolysis does not show on laked blood.

The hormone plate is perfectly transparent and affords opportunity for examination by transmitted light, but no hemolytic organisms can be detected. In addition to this disadvantage, the preparation of the medium is a difficult procedure and is not compensated for by any decided advantage over blood agar.

Workers using either whole or laked blood exclusively have reported about the same percentage of positives which would seem to indicate no appreciable difference in the efficiency of the various plates. The balance of opinion seems slightly in favor of the less opaque yet still rich whole blood plate.

In preparation of the agar base used for blood plates it has been found at the United States Naval Medical School that ordinary nutrient agar (2 to 3%) made up with Liebig's beef extract, salt, and peptone in the usual amounts gives satisfactory results. Many workers insist on meat infusion agar.

Some of the laboratories have doubled the amount of peptone; some have omitted using salt, and some have added 1 to 2% glucose.

Blood-streaked Agar

Sterilize the lobe of the ear and puncture with a sterile needle. Collect the exuding blood on a large platinum loop and smear it over the surface of an agar slant. It is advisable to incubate over night as a test for sterility. Plates or slants of glycerine agar of neutral reaction smeared with blood give the best results when such delicate pathogens as pneumococci, streptococci, gonococci or meningococci are to be cultured.

Bile Media

Secure ox bile from the abattoir or human bile from cases of gall-bladder drainage in hospitals. Put about 10 c.c. in each tube and sterilize. Some prefer to add 1% of peptone. Conradi's medium is ox bile containing 10% of glycerine and 2% of peptone. This is the medium for blood cultures in typhoid, etc.

The *bile lactose medium* now used in water analysis is made by adding 1% of lactose to ox bile and tubing in fermentation tubes. As a substitute for fresh bile one may use a 15 to 20% solution of a good quality of inspissated ox gall (Fel Bovis Purificatum). A *liver bouillon* made by using 500 grams of finely divided beef liver in 1000 c.c. of water with 1% peptone, and prepared as for meat infusion broth, is a good substitute for bile.

Rector's Bile Lactose Neutral Red Medium

This is recommended in the isolation of the colon bacillus as superior to lactose litmus agar. It consists of 10% of dried ox bile, 1% of peptone, and 1½% agar. After the medium is filtered and tubed we add 1% of lactose and 1% of a 1–100 neutral red solution. Colon colonies have a distinct purplish red zone. Furthermore the bile inhibits the growth of many organisms which give pink colonies on lactose litmus agar. MacConkeys' bile salt medium contains ½% of sodium taurocholate and is colored with neutral red.

Thalman's Medium for the Gonococcus

Five hundred grams of lean, finely minced beef are placed in 1000 c.c. of distilled water and allowed to stand over night in an ice box. It is then filtered and the filtrate made up to 1000 c.c. with distilled water. To 100 c.c. of the beef juice add 1½ grams of agar, and boil for 15 minutes. Then add 2 grams of glucose, and bring the reaction to plus 0.6 by addition of N/1 NaOH. Tube, sterilize, slant, and incubate over night. No peptone or salt is required.

Petroff's Tubercle Bacillus Medium

This is a remarkably valuable medium for isolating tubercle bacilli from sputum or pus directly. Fresh sputum should be used and for destruction of contaminating

organisms it should be shaken up with an equal amount of 3% NaOH solution and left in the incubator for one or two hours. Neutralize with N/1 HCl, using litmus paper, and centrifugalize. Take up sediment and smear out on slants of the following medium.

Treat 500 grams of chopped up meat with 500 c.c. of 15% glycerine solution. Keep in ice chest twenty-four hours and filter through gauze. Sterilize the shells of eggs by immersion in 70% alcohol for ten minutes or by dipping them in boiling water for five seconds or so. Mix white and yolk of these eggs in a sterile mortar and add an equal volume of the glycerine meat infusion which should have added to it before mixing 1 c.c. of 1% alcoholic solution of gentian violet to each 100 c.c. of the glycerine meat infusion.

Should one be culturing bovine strains the glycerine should be omitted from the meat infusion but the gentian violet (1–10,000) added. Put 3 to 4 c.c. of this medium in test-tubes and inspissate as slants at 85°C. until the medium is solidified. Subject these slants to temperature of 75°C. on the second and third days for one hour.

Plating Media for Fæces Work

The media of Endo, Conradi-Drigalski and the lactose litmus agar medium are probably the most satisfactory of the numerous ones that have been proposed for plating out fæces. A convenient way of preparing any one or all of these, and which apparently gives media equal to that prepared according to the original formulæ, is as follows:

Liebig's extract	5 grams.
Salt	5 grams.
Pepton	10 grams.
Agar	30 grams.
Water to make	1000 c.c.

Prepare as for ordinary nutrient agar, with the difference that the reaction should be brought down to 0. Some prefer a reaction of +0.2.

A stiff agar (3%) is employed to check the diffusion of acid beyond the colony.

For Endo's Medium

Keep this agar base in 100 c.c. quantities in Erlenmeyer flasks instead of test-tubes. (If more convenient smaller quantities may be put in the flask.) When needed for plating, melt a flask of this agar, and while liquid add to the 100 c.c. 6 drops of a saturated alcoholic solution of basic fuchsin, and then about 20 drops of a freshly prepared 10% solution of sodium sulphite. The sulphite solution decolorizes the intense red of the fuchsin to a light rose pink. This color fades to a light flesh or pale salmon color when cold. Now add 5 c.c. of a freshly prepared hot aqueous 20% solution of chemically pure lactose. If only occasionally using such media, tube in 20 c.c. quantities and add 1 drop of the basic fuchsin and 4 drops of the sodium sulphite solution and 1 c.c. of the hot freshly prepared lactose solution to a tube of the melted agar base just before pouring the plate. This

medium contains 1% of lactose. Kendall prepares an Endo medium which only contains 1½% of agar and with a reaction just alkaline to litmus (about plus 1.2%).

Colon bacilli show on this medium as vermilion colonies, which in about thirty-six hours have a metallic scum on them. Typhoid and dysentery colonies are grayish. Streptococci a deep red.

Standard Endo Media for Water Analysis.—The *Hygienic Laboratory-Endo medium* consists of a 3% agar which is titrated and corrected to +0.5 to phenolphthalein, to which is added 3.7 c.c. of a 10% solution of anhydrous sodium carbonate. For convenience it is flasked, sterilized, and stored in 200 c.c. quantities. When ready to use the following ingredients are added to 200 c.c. of agar, as follows:

(*a*) Dissolve 2 grams C. P. lactose in 25 to 30 c.c. of distilled water, with the aid of gentle heat.

(*b*) Dissolve 0.5 gram of anhydrous sodium sulphite in 10 to 15 c.c. of distilled water.

(*c*) To the sulphite solution add 1 c.c. of saturated solution of basic fuchsin in 95% alcohol.

Add the fuchsin-sulphite solution to the lactose solution, and then add the whole to the agar. Pour plates at once and, after hardening, dry for fifteen minutes in the incubator.

The *Standard Methods Endo medium* consists of a 3% agar made neutral to phenolphthalein, flasked, sterilized, and stored in convenient quantities. When ready to use, to 200 c.c. of agar there are added 2 grams of C. P. lactose and the agar is then melted in the Arnold sterilizer. A 10% solution of anhydrous sodium sulphite is prepared and to 10 c.c. of this solution 2 c.c. of a 10% solution of basic fuchsin in 95% alcohol are added, and this solution is heated for a few minutes. To the 200 c.c. of melted lactose agar is then added 1 c.c. of the fuchsin-sulphite solution. Plates are poured and, when hardened, placed in the incubator for drying.

For Lactose Litmus Agar

Color the 100 c.c. of agar base with litmus solution to a lilac color. Then add 5 c.c. of the hot freshly prepared 20% lactose solution in distilled water. This may be tubed, putting 10 c.c. in each test-tube, or put in quantities of 50 or 100 c.c. in small Erlenmeyer flasks. It is then sterilized in the autoclave (10 pounds for fifteen minutes) or in the Arnold.

For Conradi-Drigalski Medium

To 100 c.c. of lactose litmus agar add 1 c.c. of a solution of crystal violet (crystal violet 0.1 gram, distilled water 100 c.c.). The medium is then ready to put into plates. Colon colonies are pink. Typhoid and dysentery colonies, a bluish-gray.

The Teague Medium

We have formerly preferred the Endo plate for typhoid work and the lactose litmus agar when culturing fæces for dysentery bacilli. More recently we have

obtained most satisfactory results with the Teague medium. The colon colonies, after eighteen hours, are deep black and opaque while the typhoid-dysentery group show colorless, transparent colonies. After thirty-six hours the plates are not very satisfactory to work with.

The medium is prepared as follows: Nutrient agar is made in the usual way, containing 1.5% agar, 1% Witte's peptone, 0.5% sodium chloride, and 0.5% Liebig's meat extract, to the liter of distilled water. It is cleared with egg-white, placed in flasks, and sterilized in the Arnold sterilizer on three successive days. The reaction is brought to +0.8. The agar is melted and saccharose 0.5% and lactose 0.5% are added. The medium is then heated for ten minutes in the Arnold. To every 50 c.c. of the medium are added 1 c.c. of 2% yellowish eosin and 1 c.c. of 0.5% methylene blue. The mixture is shaken and plates poured. Eosin solution should be added first.

Conradi's Brilliant Green Medium

Take of Liebig's extract 20 grams (2%), peptone 10 grams (1%), agar 30 grams (3%) and water to 1000 c.c. This amount of meat extract should give about the proper acidity, +3. If not, the reaction should be adjusted to that point. Filter through cotton, tube 150 c.c. amounts into 250 c.c. Erlenmeyer flasks and sterilize.

Then add 1 c.c. of a 1 to 1000 aqueous solution of brilliant green (Höchst) and 1 c.c. of a 1% solution of picric acid to the flasks containing 150 c.c. of the melted agar. Sterilization after adding the dyes precipitates them and is unnecessary. Pour the finished medium into large Petri dishes and inoculate the surface with the fæces.

Brilliant green does not interfere with agglutination as does malachite green.

This medium is considered by some authorities the one of choice in isolating typhoid bacilli from fæces and urine.

The surface of the poured plates of Endo, Conradi-Drigalski, and the brilliant green media should be dried in the incubator before smearing with the fæces. For routine work I prefer Endo's medium followed by Russell's double sugar agar.

Selective Media for Cholera

Dieudonne's medium rests on the ability of cholera to grow when alkali is present in such amounts as to inhibit the growth of other fæcal bacteria.

Take equal parts of defibrinated blood obtained at the slaughter house and normal NaOH solution. Mix 30 parts of this alkaline blood mixture with 70 parts of hot 3% nutrient agar. The poured plates should be left half open over night in the incubator otherwise even cholera will not grow on the plates.

Krumwiede has as a formula for his medium equal parts of whole egg and water, to which 50% water egg mixture is added an equal amount of 12½% crystal sodium carbonate solution. This alkaline egg mixture is steamed for twenty minutes. To prepare add 30 parts of this alkaline egg mixture to 70 parts of meat extract free 3% agar. (No meat extract; only peptone and salt.) The cholera colony has a hazy look, like a little wad of absorbent cotton sticking to the surface with a metallic luster halo.

Other selective media for cholera are those of Kabeshima in which a hæmoglobin extract is treated with alkalis and added to agar.

The medium of Esch has been highly recommended. It is easy to make. Heat 500 grams chopped up beef with 250 c.c. normal NaOH solution in a pot and when disintegrated filter through cloth and sterilize. About 1 part of this alkaline extract is added to 2½ to 2 parts of agar. The plates must be dry. The transparency of this medium is an advantage.

Russell's Double Sugar Agar

A fairly stiff agar (2 to 3%) with a reaction of about +0.7 is colored with litmus solution to produce a distinct purple-violet color. It may be necessary to add more alkali. To this litmus tinted agar is added 1% of lactose and 0.1% of glucose and the medium as thus prepared is tubed and slanted. Sterilization should be carried on in the Arnold, on two successive days, as the autoclave temperatures tend to break up the sugars.

On these slants typhoid shows a delicate growth on the violet slant with a deep pink in the butt of the tube. The paratyphoids show gas bubbles in a pink butt with a violet slant.

The colon bacillus turns both slant and butt a deep pink and the butt is filled with gas bubbles. To inoculate this medium we take material from a suspicious colony grown on Endo and smear the material on the slant; then with the same platinum needle we stab into the butt. Andrade's indicator is better than litmus.

Lead Acetate Medium

This medium is useful in differentiating the paratyphoids. A paratyphoid B gives a brownish discoloration which is not given by typhoid or paratyphoid A organisms.

In its use we carry out the technic of a Russell tube, stabbing into the butt of the tube and streaking the slant.

To make: take 1½% nutrient agar to which is added 1% glucose, 1% lactose and 0.05% basic lead acetate. The basic lead acetate solution is made up as a 0.5% solution and sterilized and the requisite amount is added to each tube before slanting.

The gas production is similar to that with the Russell tube.

Culture Media for Protozoa

Medium of Musgrave and Clegg

Dissolve in 1000 c.c. of water 0.3 to 0.5 gram Liebig's extract and 0.3 to 0.5 gram of common salt. If desired for plating add 2 to 3% of agar.

A very satisfactory substitute is ordinary nutrient bouillon diluted one to ten.

Medium of Smith

Glucose 1.0 gram; Peptone 1.0 gram; NaCl 0.2; Aqua destill. 1000.0; Na_2CO_3 0.3. Agar q. s. is added for solid medium.

Medium of Castellani

This is an aqueous medium containing 1% of lactose and 10% of egg albumin This may replace water of condensation in an agar slant.

Autolyzed Tissue Media

Couret and Walker have possibly grown amœbæ from liver abscess on autolyzed tissue media. Sterile organs from healthy animals as well as human placenta are placed in sterile flasks and kept in a thermostat at 40°C. for about two weeks. The liquid from the autolyzed tissues should be about neutral and is either applied to the surface of agar slants or is mixed with melted agar. The medium is then sterilized.

Novy MacNeal Medium

Cover 125 grams of chopped up beef with 1000 c.c. of water and place over night in the refrigerator. Strain and add 20 grams of peptone, 5 grams salt, 10 c.c. of normal sodium carbonate solution and 20 to 25 grams agar. Prepare as for nutrient agar and sterilize. To 1 part of this one-quarter strength meat infusion nutrient agar, when melted and cooled down to 60°C., add twice its volume of defibrinated rabbit's blood. This medium is the standard one for the culture of certain trypanosomes and other protozoa. Under the designation N.N.N. medium (Nicolle Novy MacNeal) Nicolle has modified the medium so that there is only salt and agar in the base to which the blood is added instead of one containing meat extract and peptone. It is the Hb which seems essential in the culture of various protozoa. Rogers used citrated salt solution, which was slightly acidified with citric acid, in his culturing of *Leishmania* from the splenic blood of cases of kala azar. Incubation at 22°C.

Row's Hæmoglobinized Saline Medium

Take 10 c.c. blood from rabbit's heart or arm vein of man, defibrinate the blood and then add 10 volumes of distilled water to lake the cells (liberation of Hb). One volume of this laked blood solution is added to two volumes of sterile 1.2% salt solution.

Culture Media for Treponemata

I. **Noguchi** formerly first inoculated material containing treponemata into the testicle of rabbits, obtaining by this procedure a pure culture, after a few transfers to the testicles of other rabbits. He now grows the organism directly from serum from a chancre. Test-tubes 2 by 20 cm. are filled with 15 c.c. of a medium consisting of 2 parts of 2% slightly alkaline agar to which when melted and cooled down to 50°C. is added 1 part of ascitic or hydrocele fluid. At the bottom of the medium in the tube is placed a fragment of fresh sterile tissue, preferably a piece of rabbit's kidney or testicle. After the medium solidifies a layer of sterile paraffin oil is run in so that it covers the solid medium to a depth of 3 cm. The material is inoculated at the bottom of the tube with a capillary pipette. Incubation at 37°C. is carried

on for two weeks. The tissue acts by removing any oxygen that may be present in the depths of the medium. Anaerobiosis is a necessary condition. Many specimens of ascitic fluid are unsuited. The tubes of Noguchi and Bronfenbrenner are shown in Fig. 7. Bronfenbrenner uses a 1½% agar instead of the 2% used by Noguchi.

M'Leod and Soga have simplified Noguchi's method as follows: Take a test-tube and fit a perforated rubber stopper which can be pushed down the tube. A piece of glass tubing is passed through the stopper to project slightly into the test-tube. The other end of the glass tube is drawn out into a capillary tube and bent over at an acute angle. The test-tube is filled to ½ or ⅔ of its depth with neutral bouillon. This is freshly boiled and when cool a piece of sterile tissue is dropped in. A strip of sterile gauze is drawn through a glass bead and soaked in the material it is desired to culture and dropped into the bottom of the tube alongside the fragment of sterile tissue. Ascitic fluid is then run in to a point which would be reached by the bottom of the rubber stopper. As quickly as possible push in the stopper and when the fluid appears in the capillary tube seal off the end in a small flame. Material for study can be obtained afterward by breaking off the capillary tip and introducing a capillary pipette.

II. Serum Agar of Muhlens and Hofmann.—Fill sterile test-tubes one-third full with horse serum. This is sterilized on three successive days at 55°C. Then add an equal amount of a 3% agar containing 0.5% glucose which has been melted down and cooled to 50°C. The mixed serum agar is then kept at 55°C. for two hours. Such tubes are inoculated as for ascitic agar rabbit tissue media and incubated under anaerobic conditions, preferably in a flask from which the air has been exhausted and the remaining oxygen absorbed as shown in the anaerobic bottle described and illustrated in Fig. 9.

CHAPTER III

STAINING METHODS

In order to study a bacterial or blood specimen the first essential is a properly prepared film; the matter of staining is of less importance.

The slide or cover-glass, after cleaning with soap and water or by special solutions, should be polished with a piece of old linen. If a glass surface is free of grease a loopful of water will smear out evenly and over the entire surface. The only quick practical way to make the slide or cover-glass grease free is to burn the surface for a moment in a Bunsen or alcohol flame. The cover-glass must not be warped. To make a preparation, apply a small loopful of distilled water on the slide or cover-glass and, touching a colony with a platinum needle, stir the transferred culture into the loopful (not drop) of water. The mistake is almost invariably made of taking up too much bacterial growth. Fluid cultures do not need dilution. Smearing the mixture over a large part of the cover-glass or over an equal area of a slide, it is allowed to dry. If very little water is used, the preparation dries readily. Otherwise it can be dried in the fingers high over a flame. As soon as dry, the cover-glass should be passed three times through the flame, film side up, to fix the preparation. Slides may be fixed by passing them five times through the flame, but the method by burning alcohol recommended for fixing blood-films gives more satisfactory bacterial fixation. For routine work the stain recommended is a dilute carbol fuchsin. Drop about 5 to 10 drops of water on the cover-glass, then add 1 drop of carbol fuchsin. Allow the dilute stain to act from one to two minutes, then wash in water, dry between small squares of filter-paper (4×4 inches), and mount in balsam or the oil used for the $\frac{1}{12}$-inch immersion objective. Löffler's methylene blue is equally good as a stain.

If cell granules are wanted, fix with alcohol, osmic acid, etc.

If only nuclei and cell outlines are desired, so that bacterial inclusions may be distinct against a perfectly clear back-ground, fix by flaming with alcohol, firing immediately after flooding.

By far the best mounting medium is liquid petrolatum. This not only has the advantage of always being of proper consistence for mounts, as opposed to Canada balsam, which must frequently be made thinner with xylol, but it is less sticky and does not develop the acidity which causes balsam mounts of Romanowsky stains to fade. Furthermore, it has superior optical qualities. It is also applicable for mounting small insects and sporangia of moulds. For permanent preparations the

border of the cover-glass should be sealed with gold size or some other cement. Some prefer to mount directly in water without preliminary drying. It is good practice to make a rule to always keep the smeared side of the preparations up—never allowing it to be reversed. By this simple rule, preparations can be carried through the most complicated staining methods without the necessity of scratching the cover-glass, etc., to see which is the film side. In grasping a cover-glass with a Cornet or Stewart forceps, be sure that the tips are well by the margin of the glass, otherwise the stain will drain off. In staining with slides, the grease pencil and the glass tubing, as recommended under Blood Smears, will be found useful. The dilute carbol fuchsin and Löffler's methylene blue are probably the best routine stains. As a rule better preparations are obtained with dilute stains than with more concentrated ones.

Löffler's Alkaline Methylene Blue.—Saturated alcoholic solution of methylene blue, 30 c.c.; 1 to 10,000 caustic potash solution, 100 c.c. (Two drops of a 10% solution KOH in 100 c.c. of water makes a 1 : 10,000 solution.)

Carbol Fuchsin (Ziehl-Neelsen).—Saturated alcoholic solution basic fuchsin, 10 c.c.; 5% aqueous solution carbolic acid, 100 c.c.

Gram's Method.—The most important staining method in bacteriological technic and the one so rarely giving satisfactory results to the inexperienced is Gram's stain. In using this method, the following points must be kept in mind:

1. Laboratory cultures (subcultures) which have been carried over for years frequently lose their Gram characteristics.

2. Cultures which are several days old or dead or degenerated do not stain characteristically.

3. The aniline gentian violet deteriorates when exposed to light in two or three days—it should be kept in the dark. It should have a rich, creamy, violet appearance.

4. The iodine solution deteriorates and becomes light in color. It should be of a rich port-wine color.

5. The decolorizing with 95% alcohol should stop as soon as no more violet stain streams out. This is best observed over a white background, washing at intervals. Do not confuse stain on forceps for that on preparation.

6. The preparation should be thin and evenly spread. Some prefer carbol gentian violet to aniline gentian violet. (Saturated alcoholic solution of gentian violet, 1 part; 5% aqueous solution of carbolic acid, 10 parts.) This tends to over stain.

The formula for aniline gentian violet is 1 part of saturated alcoholic solution gentian violet and 3 parts of aniline oil water (made by adding 2 c.c. aniline oil to 100 c.c. distilled water, shaking violently for three to five minutes and then filtering several times to get rid of the objectionable oil droplets which, in a Gram-stained preparation, show as con[illegible]ing black dots).

The following stock solutions of Weigert are recommended:

No. 1		No. 2	
Gentian violet.....	2 grams.	Gentian violet...	2 grams.
Aniline oil.....	9 c.c.	Distilled water...	100 c.c.
Alcohol (95%).....	33 c.c.		

These stock solutions keep indefinitely. Mix 1 c.c. of No. 1 with 9 c.c. of No. 2. Filter. This keeps about two weeks and is the solution to pour on the preparation. It may be kept on from two to five minutes. Some hasten the staining by steaming as for tubercle bacilli. Next wash the preparation with water and flood the cover-glass with Gram's iodine solution. Some bacteriologists simply pour off excess of aniline gentian violet and immediately drop on the iodine solution. It is well to repeat the application of the iodine solution a second time. The iodine solution is left on one minute or until the preparation has a coffee-grounds color.

GRAM'S IODINE SOLUTION

Iodine.....	1 gram.
Potassium iodide.....	2 grams.
Distilled water.....	300 c.c.

After washing off the excess of iodine solution at the tap, drop on 95% alcohol and decolorize until no more violet color streams out. Now wash again and counterstain either with the dilute carbol fuchsin or with a saturated aqueous solution of Bismarck brown. For the Bismarck-brown counterstain dissolve 0.2 gm. in 100 c.c. of boiling water. Cool and filter. For the diluted carbol fuchsin stain use 1 part of carbol fuchsin to 10 parts of water. The safranin counterstain is described under Sterling's gentian violet. The Gram-negative organisms are stained brown or reddish.

The Gram-positive bacteria are stained a deep violet.

In staining smears of pus for gonococci or other Gram-negative bacteria it is best to first stain with the gentian-violet solution for two to five minutes. Then wash and examine the preparation mounted in water. The organisms stand out prominently. After noting the presence of the cocci treat the smear with the Gram solution and proceed as in the usual Gram staining technic.

Sterling's Gentian Violet.—Five grams of gentian violet are ground in a mortar with 10 c.c. of 95% alcohol. After practical solution, 2 c.c. of aniline oil are added and then 88 c.c. of distilled water. The grinding is continued a short time and after the mixture is permitted to rest a day or two, it is filtered through paper. It has the merit of staining quickly and intensely and of keeping many months.

The film, air-dried and passed through the flame, or preferably fixed in methyl alcohol, is stained as follows: The gentian violet is applied for one-half minute and washed or blotted off. The Gram's solution is applied also for one-half minute and after washing or blotting away the excess, the slide is immersed in a Coplin jar containing 95% alcohol for a few seconds. It is advisable to move it up and down. After washing away the alcohol, the counterstain, consisting of aqueous safranin is put on for one-half to one minute or longer. It does not overstain. (The safranin stain

is made by dissolving water-soluble safranin in 95% alcohol to saturation. Ten cubic centimeters of the alcoholic solution are mixed with 90 c.c. of distilled water. The stain keeps.)

Stained by Gram's Method	Not Stained by Gram's Method
S. pyogenes aureus.	Meningococcus.
S. pyogenes albus.	M. catarrhalis.
S. pyogenes.	M. melitensis.
M. tetragenus.	B. typhosus.
Pneumococcus.	B. coli communis.
Anthrax bacillus.	B. dysenteriæ (Shiga).
Tubercle bacillus.	Sp. choleræ asiaticæ.
Lepra bacillus.	B. pyocyaneus.
Tetanus bacillus.	B. mallei.
Diphtheria bacillus.	B. pneumoniæ (Friedländer).
B. aerogenes capsulatus.	B. proteus.
Oidium albicans.	B. of influenza.
Mycelium of actinomyces.	B. of bubonic plague.
Saccharomyces.	B. of chancroid.
Hofmann's bacillus.	B. of Koch-Weeks.
B. xerosis.	Gonococcus.

Practically all pathogenic cocci are Gram-positive, except the *Gonococcus*, the *Meningococcus*, the *M. catarrhalis*, and the *M. melitensis*.

Practically all pathogenic bacilli are Gram-negative, except the spore-bearing ones, the acid-fast ones, diphtheria and diphtheroid organisms.

The bacillus of glanders is Gram-negative.

Method for Staining Acid-fast Bacilli.—1. Carbol fuchsin, with gentle steaming for three to five minutes or in the cold for fifteen minutes.

2. Wash in water.

3. Decolorize in 95% alcohol containing 3% of hydrochloric acid (acid alcohol), until only a suggestion of pink remains—almost white.

4. Wash in water.

5. Counterstain in saturated aqueous solution of methylene blue or with Löffler's methylene blue.

6. Wash, dry, and mount.

The steaming of the slides with carbol fuchsin is most conveniently carried out by resting the slides on a piece of glass tubing bent into a V or U shape.

The **Leprosy Bacillus** is usually considered as being rather easily decolorized by alcohol. It is therefore often recommended to use 20% aqueous solution of sulphuric acid or nitric acid for decolorization instead of the acid alcohol above recommended for tubercle bacilli. I have often found the leprosy bacilli as resistant to

alcohol as tubercle bacilli. The smegma bacillus, however, easily decolorizes with the acid alcohol and in a well-decolorized smear from urinary sediment one can usually feel sure that any acid-fast bacilli are tubercle bacilli.

Fontes Method.—A method in which the organisms or granules which stain by the Gram method, and to which so much importance is attributed by Much, may be stained, as well as those retaining acid-fast properties, has been proposed by Fontes. The method is to stain the preparation with carbol fuchsin, decolorize with acid alcohol, then carry through the various steps of the Gram method, counterstaining, however, with Bismarck brown. Fontes in his method used 1 part of absolute alcohol and 2 parts of acetic acid as the decolorizing agent. I have obtained, however, just as satisfactory results with the acid alcohol. By this method the acid-fast tubercle bacilli show as red rods dotted with violet granules. Those which do not fully retain acid-fast properties show as zigzag violet lines.

Herman's Stain for Tubercle Bacilli.—It has been claimed that this stain gives better satisfaction than the Ziehl-Neelsen. It consists of two solutions: (1) ammonium carbonate in distilled water, 1%; (2) crystal violet (methyl violet 6B) in 95% ethyl alcohol, 3%. The two solutions are kept in separate bottles and, for staining, 1 part of (2) is mixed with 3 parts of (1). The sections are placed on a cover-glass, the water evaporated, and about 7 drops of the staining mixture are placed on the specimen and allowed to steam for one minute over a water-bath. Place for a few seconds in 10% nitric acid and then in 95% alcohol to decolorize. Mount without a counterstain or use eosin 1% or a very dilute fuchsin. The organisms are purple. This staining method may be applied to smears of concentrated or unconcentrated sputum in the same manner as for sections of tissue.

Smith's formol fuchsin:

Saturated alcoholic solution basic fuchsin	10 c.c.
Methyl alcohol	10 c.c.
Formalin	10 c.c.
Distilled water to make	100 c.c.

This gives a very sharp differentiation of bacteria and nuclear structures. It has a purplish tinge. Fixation by heat gives the best staining. Allow the stain to act for two to ten minutes. It should not be used until after standing twenty-four hours, and after standing about two weeks it appears to lose its sharp staining power.

Archibald's Stain.—This is an excellent bacterial stain and has been highly recommended by Blue and McCoy in plague work.

Solution No. 1		Solution No. 2	
Thionin	0.5	Methylene blue	0.5
Phenol crys	2.5	Phenol crys	2.5
Formalin	1.0	Formalin	1.0
Water	100.0	Water	100.0

Dissolve for twenty-four hours. Mix equal parts and filter. Stain smears fixed by heat or otherwise for ten seconds.

Nicolle's Carbol Thionin

Sat. sol. thionin in 50% alcohol	10 c.c.
Carbolic acid solution (2%)	100 c.c.

Pappenheim's Stain.—Take a very small portion of methylene green on the point of a penknife and shake it into a test-tube. Then take up twice as much pyronin and deposit it in the same test-tube. Fill the test-tube one-half full with water and the solution should have a distinct reddish-violet color. A drop on a piece of filter-paper shows a violet center and peripheral green ring. The solution should be fresh. Stain from two to five minutes. Differentiate with a little resorcin on a penknife point dissolved in one-quarter of a test-tube full of alcohol. Dehydrate, clear and mount. Polymorphonuclear nuclei stain greenish; nuclei of mononuclears and plasma cells from bluish-red to dull violet. Cytoplasm of lymphocytes and plasma cells purplish-red. Bacteria red.

Romanowsky Stains.—See under section on Blood. For mounting specimens showing chromatin staining, as malarial parasites, trypanosome, intestinal flagellates, etc., liquid petrolatum is to be highly recommended. The chromatin staining lasts without any fading for at least two years. The acidity of balsam causes rapid fading of the chromatin.

Neisser's Stain for Diphtheria Bacilli

Solution No. 1		Solution No. 2	
Methylene blue	0.1 gram.	Bismarck brown	0.2
Alcohol	2 c.c.	Water (boiling)	100 c.c.
Glacial acetic acid	5 c.c.	Dissolve the stain in the boiling water and filter.	
Distilled water	95 c.c.		
Dissolve the methylene blue in the alcohol and add it to the acetic acid water mixture. Filter.			

To stain: Fix the preparation. Pour on the dilute acetic acid methylene blue solution and allow to act from thirty to sixty seconds. Wash. Then pour on the Bismarck-brown solution, and after thirty seconds wash off with water. Dry and mount. The bodies of the bacilli are brown with dark blue dots at either end.

Neisser recommends only five seconds as the time of application of each solution. He also recommends that the culture be only nine to eighteen hours old and that the temperature of the incubator shall not exceed 36°C. Incubation at 37°C. gives satisfactory results.

Ponder's Stain for Diphtheria Bacilli

Toluidin blue (Grubler)	0.02 gram.
Glacial acetic acid	1 c.c.
Absolute alcohol	2 c.c.
Distilled water to	100 c.c.

The film is made on a cover-glass and fixed in the usual way. A small quantity of the stain is spread on the film and the cover-glass is turned over and mounted as a hanging-drop preparation. The metachromatic granules of the diphtheria bacilli stain with striking intensity. With diphtheroids, the more intense staining sharply differentiates from ordinary cocci and bacilli, which show in the preparation only as faint light blue bodies. It is a most excellent stain for bringing out the ascospores of yeasts. In my opinion the stain is more valuable than the Neisser method.

Capsule Staining.—The best method for studying bacteria, as to presence of capsules, is in the hanging drop, with the greater part of the light shut off by the diaphragm.

In material where capsules are well developed, as in pneumonic sputum, the Gram method of staining brings out the capsule perfectly. This is of diagnostic value, as the more or less nonpathogenic pneumococci common about the mouth do not seem to show a capsule when stained in this way. The India ink method of staining gives good results for capsules.

The most beautiful method of staining capsules is the latest one proposed by Muir.

1. Prepare thin film, dry and stain in carbol fuchsin one-half minute; the preparation being gently heated (steamed).
2. Wash slightly in 95% alcohol, then wash well afterward in water.
3. Flood preparation in mordant for five to ten seconds.

Mordant.—Sat. aqueous sol. mercuric chloride..... 2 parts.
Tannic acid (20% aqueous sol.)..... 2 parts.
Sat. aqueous sol. potash alum..... 5 parts.

4. Wash in water thoroughly.
5. Treat with 95% alcohol for one minute. (The preparation should have a pale red color.)
6. Wash well in water.
7. Counterstain with methylene blue one-half minute.
8. Dehydrate in alcohol. Clear in xylol and mount. (May simply dry specimen with filter-paper.)

Rosenow's Capsule Stain.—Make a very thin smear of the pathological material and when nearly dry cover the preparation for ten to twenty seconds with 10% tannic acid solution. Wash in water and blot. Stain with aniline gentian violet by gently steaming for one-half to one minute. Wash in water. Apply Gram's

iodine solution for one-half to one minute. Decolorize in 95% alcohol and then stain with alcoholic solution of eosin. Wash in water, dry and mount.

Hiss' Capsule Stain.—Preparations are best made by direct films from pneumococcus exudates. Dry in air and fix by heat. Stain for a few seconds with saturated alcoholic solution of fuchsin or gentian violet, 5 c.c., in distilled water, 95 c.c. Flood the slide with the dye and hold the preparation for a second over a free flame until it steams. Wash off the dye with 20% aqueous copper sulphate solution. Blot (do not wash in water).

By this method the capsule appears as a faint blue halo around a dark purple cell body. Better results may frequently be obtained by omitting heat fixation and by washing off the dye with the copper sulphate solution as soon as it begins to steam. Water should not be applied at any stage of the procedure.

Flagella Staining.—Inoculate a tube of sterile water (gently) in upper part, with just enough of an eighteen to twenty-four-hour-old agar culture to produce faint turbidity. Incubate for two hours at 37°C. From the upper part of culture take a loopful and deposit it on a cover-glass. Dry in thermostat for one to five hours or over night. Use perfectly clean cover-glasses. To stain by

Muir's Modified Pitfield Method

1. Flood specimen with mordant. Steam gently one minute.
   Mordant.—Tannic acid (10% aqueous solution)..... 10 c.c.
   Sat. aq. sol. mercuric chloride..... 5 c.c.
   Sat. aq. sol. alum..... 5 c.c.
   Carbol fuchsin..... 5 c.c.
   Allow precipitate to settle or centrifuge. Keeps only one week.
2. Wash well in water for two minutes.
3. Dry carefully—preferably in incubator.
4. Pour on stain. Steam gently one minute.
   Stain.—Sat. aq. sol. alum..... 10 c.c.
   Sat. alc. sol. gentian violet..... 2 c.c.
   (May use carbol fuchsin instead of gentian violet.)
   Stain only keeps two days.
5. Wash well in water. Dry and mount.

Zettnow's Flagella Staining Method

Solution I.—Dissolve 2 grams of tartar emetic in 40 c.c. water.

Solution II.—Dissolve 10 grams tannin in 200 c.c. water. To the 200 c.c. solution II, warmed to 50 or 60°C., add 30 c.c. of the tartar emetic solution. The turbidity of the mordant should entirely clear up on heating. The mordant should keep for months when a small crystal of thymol is added to it.

Next dissolve 1 gram silver sulphate in 250 c.c. distilled water. Of this solution take 50 c.c. and add to it drop by drop ethylamine (this comes in a 33% solution)

until the yellowish-brown precipitate which forms at first is entirely dissolved and the fluid is entirely clear. It requires only a few drops. The bacterial preparations prepared as described above are floated in a little mordant contained in a Petri dish which is heated over a water-bath for five to seven minutes. Take the dish containing the preparation off the water-bath and as soon as it becomes slightly opalescent as the result of cooling remove the cover-glass preparation and wash thoroughly in water. Then heat a few drops of the ethylamine silver solution upon the mordanted cover preparation until it just steams and the margin appears black. Next wash thoroughly in water and mount. This gives the most satisfactory results of any method I have ever experimented with.

Spore Staining.—The most satisfactory spore staining method is really the negative staining of the spore obtained when a bacterial preparation is stained by dilute carbol fuchsin or Löffler's methylene blue. The spore appears as a highly refractile piece of glass in a colored frame.

The acid-fast method, as for tubercle bacilli, gives good results. The decolorizing, however, must be lightly done, otherwise the spore will lose its red stain.

Möller's Method.—Fix films and then treat with chloroform for one or two minutes. Wash thoroughly and treat with a 5% solution chromic acid for one minute. Wash in water and then stain as for acid-fast organisms with carbol fuchsin. Use a 1% sulphuric acid solution instead of the 3% acid alcohol.

Agar Jelly Staining Method of H. C. Ross

Very clear 1½% solution of agar is colored with Unna's polychrome methylene blue, Giemsa's solution, thionin or Gram's solution of iodine. Very thin smears of blood, fæces or gastric content sediment are made and either fixed lightly in the flame or air dried. A drop of the melted colored agar solution is placed on the smeared cover-glass and this is mounted immediately on a clean slide. The preparation is ready for examination in about two minutes.

The Staining of Protozoa

Unless staining albuminous material it is well to add a little blood-serum albumin fixative or white of egg to the preparation—about one loopful to a smear. The serum or white of egg is best preserved by the addition of 2% chloroform and kept tightly corked.

Giemsa's Method.—Fix moist smears with a fixative made by adding 1 part of 95% alcohol to 2 parts of saturated aqueous solution of bichloride of mercury. Keep in this solution 1 to 12 hours. Now wash for a few seconds in water and then for about five minutes with a dilute Lugol's solution (KI, 2 gm.; Lugol's solution, 3 c.c.; Aqua, 100 c.c.). Now wash in water and then in a 0.5% solution of sodium thiosulphate to remove the iodine which was used to remove the mercury. Wash in water five minutes, then stain with Giemsa's stain as used in blood-work for one to ten hours. Wash and mount.

Vital Staining of Protozoa with Neutral Red Solution.—As a stock solution one uses a 0.5% aqueous solution of neutral red.

The drop of salt solution or water on the slide should be tinged a light violet-rose color with a fraction of a loopful and the fæces or other material emulsified in this.

Protozoa take a rose-pink color with a distinct differentiation between endoplasm and ectoplasm.

Should the fæces be quite alkaline the neutral red will be decomposed with the formation of bilirubin-like crystals.

The Giemsa formalin method described under Blood-work is of value in certain cases.

Panoptic Method.—Highly to be recommended for the staining of protozoa, whether in smears or in sections, is the Panoptic method.

1. Wright's or Leishman's stain for one minute.

2. Dilute with water and allow dilute stain to act for three to ten minutes. Wash in water and then

3. Pour on dilute Giemsa's stain. Allow to stain from thirty minutes to twenty-four hours. Differentiate with 1 : 1000 acetic acid solution until blue stain just shows commencing diffusion into the acetic acid. Then wash in water, 95% alcohol, absolute alcohol and treat with xylol and mount in liquid petrolatum.

With preparations other than blood smears, as sections, it is better to go from 95% alcohol to oil of origanum, then mount.

Owing to the great value of a sharp nuclear picture in differentiating amœbæ it is of great importance to use some iron hæmatoxylin method. That of *Weigert* is given in the appendix.

Mallory's Phosphotungstic Hæmatoxylin.—Fix moist smears, film surface down, in Zenker's fluid for five to ten minutes. Wash in water, treat with Gram's solution and wash with 70% alcohol until all the yellow color is discharged. Wash in water. Then stain with *Mallory's phosphotungstic hæmatoxylin* for one-half hour. Wash, clear and mount. See appendix.

Mallory's Differential Stain for Amœbæ.—Staining in saturated aqueous solution thionin for from three to five minutes. Next differentiate in 2% aqueous solution oxalic acid for one-half to one minute. Then wash in water, clear and mount. Nuclei of amœbæ are stained a brownish red.

Rosenbusch Iron Hæmatoxylin Stain

Rapidly smear out with a toothpick a small particle of fæces or other material containing protozoa and, while still moist, fix by Giemsa's method and, after getting rid of the mercury with iodine followed by 95% alcohol, treat smears with a 3.5% solution of iron-alum in distilled water for one-half hour or over night, then wash thoroughly in distilled water.

Then stain from five to twenty minutes in the following hæmatoxylin stain: (1) 1% solution of hæmatoxylin in 95% alcohol. It takes at least ten days to ripen. (2) A saturated solution of lithium carbonate. Add to 10 c.c. of the hæmatoxylin

solution 5 to 6 drops of the lithium carbonate one. Next wash well and differentiate with about a 1% solution of the iron alum. Again wash in water, pass through alcohols to xylol and mount in balsam.

Mallory's Iron Hæmatoxylin Method

I have obtained beautiful staining with this simple method. The great point in technic is the watching of the differentiation.

Treat sections or moist smears fixed by Giemsa's method with 10% aqueous solution of ferric chloride for three to five minutes. Then drain off iron solution, blot the section and stain for four minutes with a freshly made solution of 1% hæmatoxylin in water. To make this add a few small crystals to 4 or 5 c.c. water in a test-tube and dissolve by heat. The stain deteriorates after twenty-four hours. Wash in water. Differentiate with a ¼% aqueous solution of ferric chloride. The differentiation is complete in from a few seconds to one or more minutes according to depth of staining and thickness of film. Wash in water, pass through alcohols and xylol and mount.

Spirochæte Staining Method (Fontana)

The smears must be air dried, not fixed by heat. Cover films with Huge's fluid which is 1 c.c. acetic acid, 20 c.c. formalin and 100 c.c. distilled water. Flood films several times with this fluid. Wash in water and cover with a mordant of 5% tannic acid in 1% carbolic acid solution. Heat the mordant on the slide until steam arises, and allow the heated mordant to act about thirty seconds. Wash in water and without drying pour on the silver stain, heat until steam arises, leave heated stain on for thirty seconds, wash in water, blot, dry and examine with immersion objective. The treponemata are brownish to black.

To make the silver stain make a ¼% solution of silver nitrate in distilled water. Then add drop by drop ammonia until a slight turbidity is produced; only a trace of ammonia is required and any excess again clears up the solution and makes it useless.

In staining spirochætes with the Giemsa or Wright stain one obtains more intense staining by using a 1% sodium carbonate solution instead of distilled water for the diluting fluid.

CHAPTER IV

STUDY AND IDENTIFICATION OF BACTERIA—GENERAL CONSIDERATIONS

In order to study bacteria it is necessary to isolate them in pure culture. This may be accomplished by taking one or more loopfuls of the material and mixing it in a tube of melted agar or gelatin. From this first tube one or more loopfuls are transferred to a second tube of melted agar or gelatin, and from this a third transfer is made, thereby giving us tubes in which the distribution of the bacteria is one or more hundred times less in the second than in the first tube, and equally more dilute in the third than in the second. When we pour the contents of the tubes into Petri dishes we would have the bacterial colonies on the first plate so thick that it would be impossible to pick up a single colony with a platinum needle without touching an adjacent one. On the second plate the distribution might be such that we should have discrete, well separated colonies, material from which could be taken up on the point of the needle or loop without touching any other colony. If the second plate did not meet these requirements, the third would.

In clinical bacteriology we work almost entirely with organisms preferring blood-heat temperature, hence it is necessary to use agar or blood agar as standard media for the obtaining of isolated colonies. Gelatin is of little value for this purpose in medical work. In using agar it will be remembered that it solidifies at a temperature slightly below 40°C. and does not melt again until it is subjected to a temperature practically that of boiling. Again, if the temperature of the media exceeds 44°C. it may affect injuriously the organisms we wish to study. Consequently it requires careful attention and quick work to inoculate the tubes, mix, transfer and pour into plates within the limits of a temperature which injures the organisms, and one which brings about the solidification of the agar.

Again, we not only have colonies developing from organisms which have been fixed at the surface as the agar solidified in the plate, but more numerous ones developing from bacteria caught in the depths of the media. Therefore we have superficial and deep colonies. Except to the person of great experience, all deep colonies look alike and there

is at times great difficulty in deciding whether a colony is deep or superficial. It is in the matter of trying to obtain information from the differences in deep colonies that the greatest difficulties in the study of bacteriology arise. By using the method of simply stroking plates

FIG. 10.—Petri agar plate. Made by spreading scrapings from the mouth over sterilized nutrient agar; after forty-eight hours in the thermostat the light "colonies" develop. Streaked plate. (*Delafield and Prudden.*)

along five or six parallel lines from one side of the plate to the other with a bent glass rod, platinum loop, or a small cotton swab, we obtain colonies which are well separated and which are entirely superficial. We pour about 10 c.c. of agar, blood agar or Endo media into Petri dishes and keep them in the refrigerator for immediate use.

Smeared or Stroked Poured Petri Plates.—The material as pus, fæces, throat membrane, etc., should be evenly distributed in a tube of sterile water or bouillon; the swab which was originally used for obtaining the material being then pressed against the sides of the test-tube to express excess of fluid and then stroked gently over successive lines on one plate. Or, if the organisms be very abundant, over a second plate without recharging it from the inoculated tube. With such material as that adhering to the loop of a nichrome wire applicator, after scraping along the posterior pharynx of a meningococcus carrier, it is satisfactory to touch the surface of the blood agar plate to deposit some of the mucoid material. Then with a bent glass rod we rub it over the entire surface of the plate revolving the plate while drawing back and forth the smearing rod.

A nichrome wire loop, somewhat bent over and having a flattened-out loop end, the weight of the handle of the nichrome wire being supported by the fingers so as not to exert pressure and cut the surface of the medium, makes a good spreader.

According to my experience a very satisfactory method is to take a loopful from the bouillon tube suspension of the pus or fæces and deposit the fluid in the platinum loop on the left half of the poured plate then, without recharging the loop, we touch the right half of the plate. Now taking a bent glass rod from a jar of 95% alcohol we flame it and to cool the same we press the bent portion into the middle of the plate. This also divides the surface of the plate into two portions. Then rubbing the bent rod over the smaller amount of the material on the right side we carry it over the entire right side. Then go to the loopful deposited on the left side with the rod and rub it over this side. For urine, deposit 1 drop on one side and 5 drops on the other. A smear from pus, sputum, urine or throat culture should always be made first in order to get an idea as to the degree of dilution which is necessitated before plating out.

We use blood agar plates as routine ones when we culture from throat, glands, joints, pus, blood, etc. The lack cf translucency does not interfere when we study the morphology of superficial colonies, using a hand lens. Then the hæmolytic zone of *S. pyogenes* or the green of *S. viridans* or the *Pneumococcus* make such a medium indispensable. All pathogens grow well on it. For fæces we use Endo's medium.

Esmarch's Roll Culture Tubes.—Having melted about 5 c.c. agar in a test-tube we inoculate the melted medium at 45°C. and very quickly roll the tube in a groove melted out of a block of ice. The agar sets on the sides of the tube and colonies may be studied with a glass. Such tubes form a large amount of water of condensation which aids in the study of streptococci. Larger tubes with rolled media are useful for culturing bacterial growth for vaccines, this technic giving a larger surface than the slant.

To obtain isolated colonies on blood-serum or blood-streaked agar, which can be touched and by transfer obtained in pure culture, we simply smear the material on a slant of either medium. Then, without

sterilizing the loop, we smear it thoroughly over a second slant, and so on to a third, or possibly a fourth or fifth.

Classification.—At present the classification of the bacteria is very unsatisfactory from a scientific standpoint. The nomenclature abounds in instances where three or four terms are used in naming a single bacterium, instead of the single generic name and single specific one as is used in zoölogical nomenclature. This matter of nomenclature is a subordinate factor in the confusion when we begin to investigate and find that different names have been applied to apparently the same organism.

The slightest variation in morphological, locomotor, or biological characteristics seems to be considered sufficient by many observers to justify the description of a new species, and, of course, the giving of a new name. Many of these names which are now retained were applied prior to the epoch-making introduction of gelatin media by Koch (1881) and consequently at a time when the isolation of organisms in pure culture was a matter of extreme difficulty and uncertainty.

In the keys to follow the term bacterium has been used as a general designation for all schizomycetes. Migula calls motile rod-shaped organisms bacilli, and nonmotile ones bacteria. Lehmann and Neumann call spore-bearing organisms bacilli, and nonspore-bearing ones bacteria.

The *B. typhosus* is very motile and does not possess spores. According to Migula, it would be the *Bacillus typhosus;* according to Lehmann and Neumann, the *Bacterium typhosum*. The *B. anthracis* has spores and is nonmotile. Hence it would be *Bacterium anthracis*, according to Migula, and *Bacillus anthracis*, according to Lehmann and Neumann.

In the use of the keys at the head of each group of organisms it will be observed that the primary separation is on the basis of morphology—the cocci in one group, the bacilli in three subgroups: one for those rod-shaped organisms showing branching and curving forms, one for the spore bearers and one for the simple rods. The spirilla are grouped by themselves.

In a study of the subject of bacterial classification by the Society of American Bacteriologists the rod-shaped organisms are classified under the families Bacteriaceæ, Lactobacillaceæ and Bacillaceæ. The Bacillaceæ are rod-shaped, Gram-negative organisms without endospores and include the genera *Bacterium* (type species *B. coli*), *Pasteurella* (type species *P. choleræ-gallinarum*) and *Hemophilus* (type species *H. influenzæ*). The Lactobacillaceæ are long, slender, nonmotile rods, Gram positive and without endospores. There is one genus *Lactobacillus*, with *L. caucasicus* as type species. The Bacillaceæ are rods producing endospores and having two genera, *Bacillus* (type species, *B. subtilis*) and *Clostridium* (type species *C. butyricum*). In the genus *Bacillus* the rods are not much changed at sporulation; with *Clostridium* the rods enlarge at that time forming clostridium or plectridium forms. The genera

Mycobacterium (*M. tuberculosis*) and *Corynebacterium* (*C. diphtheriæ*) have the characteristics noted in the keys to follow. These organisms belong to the family Mycobacteriaceæ. The round forms are in the family Coccaceæ, with the following type species for the composing genera: *Neisseria gonorrhœa, Streptococcus pyogenes, Staphylococcus aureus, Micrococcus luteus* and *Sarcina ventriculi.*

Under the family Spirillaceæ we have the genera *Vibrio* (type species *V. choleræ*) and *Spirillum* (no type species given).

Motility.—One of the first facts noted by the student in taking up bacteriology is the difficulty in determining motility; this property should always be tested on young cultures in bouillon. In Brownian movement there is a sort of scintillating movement, but the bacterium does not move from that part of the field. In current movement all the bacteria swarm in the same direction, going very fast at times, and then more slowly.

If in great doubt, the mounting of the organisms in a 2% solution of carbolic acid will stop movement if it be true functional motility, while Brownian and current movement are not interfered with. In true motility bacteria move in opposite and in all directions, and move away from the place where first observed unless degenerated or dead.

At times we judge of motility by the presence of this characteristic in a few of the organisms seen in the microscopic field, the vast majority of the bacteria not showing motility. A source of error can be present when the bacteria are emulsified in a drop of water which might contain motile bacteria.

To determine motility young eighteen-hour-old bouillon cultures are preferable, and the preparation should be made by applying a vaseline ring to the slide, then putting a drop of the bouillon culture in the center of the ring (or a drop of water inoculated from an agar slant growth), then putting on a cover-glass. By this method current movement is done away with and the preparation keeps for hours. This is a convenient method for agglutination tests. The hanging drop with a concave slide is ordinarily used. With this, cut down the light and focus on the margin of the drop with the ⅔-inch objective before examining with a high dry objective (⅙ inch).

Reaction of media is a factor of the greatest importance in causing variation in the functions and even morphology of bacteria, and is one which has until recently been almost entirely neglected. In describing an organism at the present time it is always necessary to note the reaction of the media, the temperature at which cultivation took place, and the age of the culture when examined.

Gram Staining.—An important method of differentiation is the reaction to Gram's stain. It should be remembered that organisms

CHART FOR STUDY OF BACTERIA

Name.....Source.....Date.....

Form.....Arrangement.....

Size, length.....Breadth.....Extreme length.....

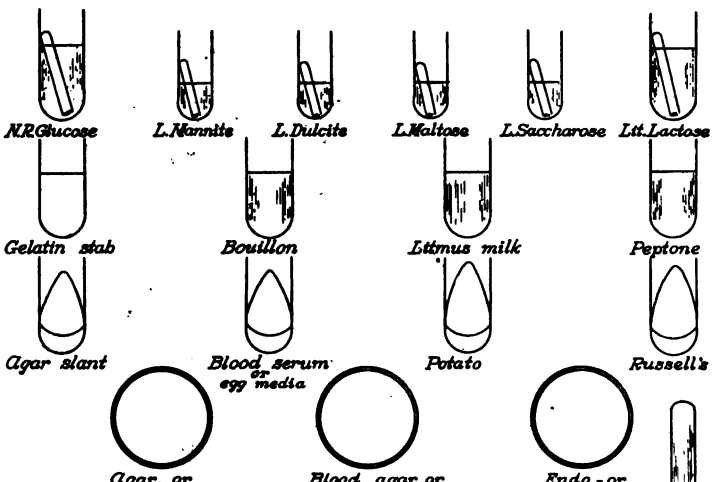
Capsules.....Spores.....Central.....Terminal.....

Motility.....Pleomorphism.....

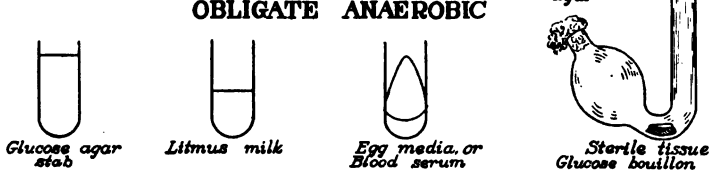
Staining, reaction, Löffler.....Gram.....Acid fast.....

Pathogenesis:- White mouse.....Guinea pig.....Rabbit.....

AEROBIC OR FACULTATIVE



OBLIGATE ANAEROBIC



Special media:.....

.....

Notes:.....

.....

.....

(E.R.M.)

FIG. 11.—Bacteriological Chart in use at the U. S. Naval Medical School. The mimeographed sheets are 8 by 14 inches. Red and blue pencil shading characterizes acid or alkaline reactions in sugar tubes. Outlines of colony in plate rings are made in pencil as is also done on slant figures.

carried along on artificial media often lose their Gram-staining characteristics; hence it is desirable to determine this staining reaction in cultures freshly isolated.

Be sure that the stains, especially the aniline gentian violet and the iodine solution, have not deteriorated. There is no more important stain than this, and none which requires greater experience. The chief causes of conflicting results are 1. working with old cultures and 2. not having satisfactory staining solutions.

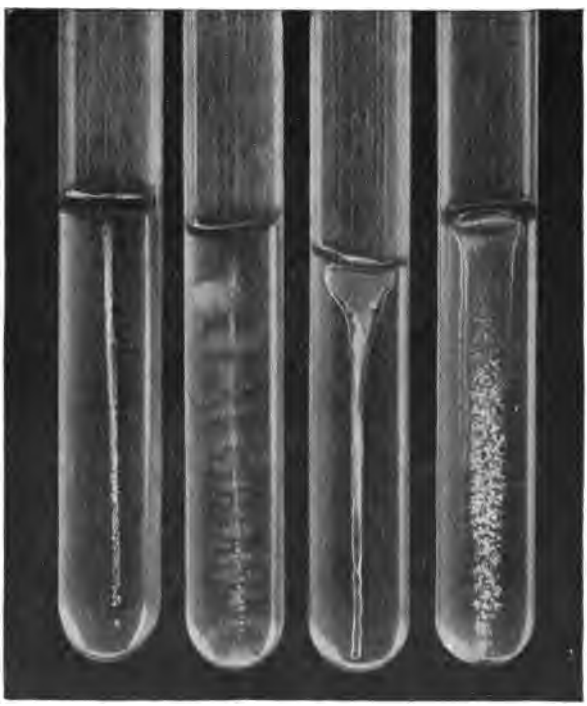


FIG. 12.—Series of stab cultures in gelatin, showing modes of growth of different species of bacteria. (*Abbott.*)

Liquefaction of gelatin is a very important means of differentiating. When room-temperature incubator is not at hand (20° to 22°C.), it is better to put the inoculated gelatin tube in the body-temperature incubator, and from day to day test the power of solidifying with ice-water. If the organism digests the gelatin (a liquefier), the medium will remain fluid when placed in ice-water; if the organism is a non-liquefier, the medium in the tube becomes solid. Of course we lose the information to be obtained from the shape of the area of liquefaction.

Acid Fermentation.—For routine work the only sugar media used are glucose and lactose bouillon. These are of the utmost importance in differentiating organisms of the typhoid and colon group. For more elaborate study we also use innumerable other carbohydrates, as maltose, saccharose, mannite, dulcite, inulin, etc. Following Ford, these intestinal bacteria have primarily been separated by their action on litmus milk—whether turning it pink or only slightly changing or not changing at all the original color.

Gas Production.—In testing for gas production it is better to use the Durham fermentation tube as small amounts of gas may not be easily detected with deep stab cultures into glucose or lactose agar.

If a Durham or Smith tube, or a slant of Russell's double sugar medium be not at hand the production of gas may be determined by observing bubble formation on the surface of the sugar bouillon culture. As none of the pathogenic cocci produce gas, fermentation tubes are unnecessary where cocci are to be studied. The litmus milk tube gives data as to acid production.

Indol production is of but slight aid in differentiating organisms. The same may be said of determining the number and disposition of *flagella* as the technic for bringing out flagella is most uncertain. *Capsule* and *acid fast* characteristic are valuable differentiating methods however.

Colony Isolation.—Examine the colonies on Petri plate at first with the unaided eye, then with a hand magnifying glass or low-power objective, using reflected and transmitted light alternately. Having determined the presence of two or more different kinds of colonies, make a ring with wax pencil around one or more of each kind of colony, numbering them. The slides or culture tubes used in determining the species of organism present in the plate should bear the same number as that of the colony from which the material was taken. A convenient procedure is to put a loopful of water on a clean cover-glass and emulsify material from a colony in it. Then invert over a concave slide without vaselining the circumference of the concavity. After examining for motility, smear out and dry the bacterial preparation. Then fix in the flame and stain with aniline gentian violet for two to five minutes. Wash and mount the preparation in water. Afterward pass through the usual Gram technic.

After this inoculate the various culture media from similar colonies. *One may inoculate a tube of bouillon from a single colony and later on inoculate the other culture tubes.*

An important point is to wait at least forty-eight hours (in the case of *M. melitensis*, four to seven days) before reporting on the cultural findings on the agar, blood agar or blood-serum slant or plate upon which the material is smeared (pus, exudate, blood, etc.).

Anaerobiasis.—If it were not for the fact that we have so many facultative anaerobes (organisms growing under anaerobic as well as aerobic conditions) it would be of practical utility to make this biological variation our first step in the study of an unidentified organism. At any rate it is well to remember that the causative organisms of plague, tuberculosis, influenza, gonorrhœa, pneumococcal pneumonia and glanders are obligate aerobes while those of tetanus, botulism, gas-gangrene and malignant œdema are obligate anaerobes. The pyogenic cocci as well as the causative organisms of cholera, typhoid, paratyphoid and anthrax are facultative anaerobes; they are, however, always studied under aerobic conditions. The colon bacillus as well as organisms of the Friedländer group are also facultative anaerobes.

Should an organism be encountered in original investigations these requirements as to etiological relationship should be carried out (**Koch's postulates**):

1. The organism should be constantly present in that particular pathological condition. 2. Such bacteria should be isolated in pure culture from the pathological material. 3. Such pure cultures when inoculated into suitable animals should reproduce the pathological conditions and should be capable of a second isolation in pure culture from such an experimental animal. For various reasons, such as unsuitable animals or artificial media, these requirements are impossible of execution with several organisms which are generally recognized as the causes of certain diseases.

Animal Experimentation

The experimental animals most frequently employed in the diagnosis of bacterial diseases are the guinea-pig, the rabbit, the white rat and the white mouse. In the following diseases the most suitable animals for inoculation are:

1. Tetanus—mice or guinea-pigs, subcutaneously. The spasms begin in the limbs nearest the site of inoculation.
2. Pneumococci and streptococci—mice, intraperitoneally, or rabbits, intravenously.
3. Staphylococci—rabbits.
4. Diphtheria, tuberculosis, anthrax and malignant œdema—the guinea-pig, subcutaneously.
5. Glanders and cholera—the guinea-pig, intraperitoneally.
6. Plague—guinea-pigs, cutaneously or subcutaneously.

In the cutaneous method of infection the material, as from a plague bubo, or the sputum from pneumonic plague, is thoroughly rubbed with a glass rod upon the shaven surface of the guinea-pig.

In the subcutaneous method one can use a hypodermic needle (the all-glass syringe with platino-iridium needle is the best) or an opening can be cut with the scissors, a pocket then opened up with the forceps and a piece of tissue inserted to the bottom of the pocket with the forceps.

One can seal the incision with collodion.

The marginal ear vein of the rabbit is used for intravenous inoculation. This can be made to stand out with either hot water or xylol.

In intraperitoneal injections the animal is best held head down so that the intestines gravitate downward. The shaven skin is pinched up and the needle inserted in the median line.

In certain cases infection is secured by feeding the material to the experimental animal. The material may be mixed with the food or introduced into the stomach by a tube.

Culturing Material Obtained at Biopsy or Autopsy of Man or Animals

It is customary to sear with a heated knife or spatula a spot on the surface of the organ from which cultures are to be made. Then introduce at this point a sterile platinum spud and inoculate tubes of culture media. The platinum loop may be used where an incision is made into the organ with a sterile knife. The ordinary platinum loop, however, bends too easily.

A bacteriological capillary pipette is a good instrument for taking up material. After some practice one can do good work with a rubber bulb capillary pipette, especially in taking up blood from right heart or blood-vessels. When great precaution is necessary, as in culturing a removed gland or organ, the piece of tissue may be dropped into 5% formalin solution for a few minutes, then washed in sterile salt solution; next placed in a sterile Petri dish and the material obtained from the center. It may be dropped for a few seconds into boiling water to sterilize the surface. When autopsying experimental animals it is well to dip the dead animal into 3% tricresol solution before opening up the body.

CHAPTER V

STUDY AND IDENTIFICATION OF BACTERIA—COCCI. KEY AND NOTES

Streptococcus Forms.—Cells divide to form chains.

I. Gelatin not liquefied.

1. Hæmolytic zone on blood agar. S. hæmolyticus (S. pyogenes).
   Very slight acidity in lactose litmus bouillon. S. pyogenes. Tends to produce arthritis in experimental animals. Often a granular sediment in bouillon.
2. Greenish appearance about colonies on blood agar.
   (*a*) No tendency to capsule formation. S. viridans. Produces endocarditis in experimental animals.
   (*b*) Distinct capsule formation in pathological material or on favorable media. S. lanceolatus (Pneumococcus). Gram-positive, lance-shaped cocci with bases apposed within a capsule.
   (*c*) Very marked capsule development on all media. P. mucosus. A streptococcus with extraordinary capsule development, up to 10μ in width, S. mesenterioides, is not pathogenic.

II. Gelatin liquefied.

Streptococcus coli gracilis. (Cocci quite small—0.2 to 0.4μ. In fæces.)
A tube-like liquefaction; chains rather long; only slight growth on agar. Constant inhabitant of stools of meat diet.

Sarcina Forms.—Cells divide in three dimensions of space. (Packets.)

A. No pigment production on agar.
   (*a*) Sarcina alba. (Colonies finely granular.)
   (*b*) Sarcina pulmonum.

B. Yellowish pigment.
   (*a*) Sarcina lutea. (Colonies coarsely granular.)
   (*b*) Sarcina flava. (Colonies finely granular.)

C. Rose-red pigment.
   (*a*) Sarcina rosea.

Micrococcus Forms.—Cells divide irregularly in various directions.

I. Gram-positive cocci.

A. Cocci-round.

1. Divide in two planes at right angles. Tetrad formation. Merismopedia.
   (*a*) M. tetragenus. Moist white viscid colonies. No liquefaction of gelatin. Capsule.

2. Divide irregularly. Bunch of grapes arrangement. (Staphylococci.)
   (*a*) Gelatin not liquefied. M. cereus albus.
   (*b*) Gelatin liquefied. { M. (Staphylococcus) pyogenes albus. / M. (Staphylococcus) pyogenes aureus.
   (*c*) Gelatin very slightly liquefied.
   S. epidermidis albus. (Stitch coccus.)

B. Cocci—biscuit-shape.
   Diplococcus crassus. (May be mistaken for Meningococcus.)

On ordinary agar we have a scanty growth resembling the Streptococcus. Colonies on ascites agar are smaller than those of Meningococcus. It produces acid in glucose, maltose, lactose and saccharose.

II. Gram-negative cocci.
   A. Grow only at about incubator temperature.
      1. Grow only on blood or serum media. Gonococcus.
      2. Grow on blood-serum media, or glycerine agar, best on blood agar.
         (*a*) Diplococcus intracellularis meningitidis. Produces acid in glucose and maltose but not in lactose nor saccharose.
      3. Grows on ordinary media. Micrococcus melitensis.
   B. Will grow at room temperature as well as at 37°C.
      (*a*) Micrococcus catarrhalis. Does not produce acid in glucose, maltose, lactose nor saccharose.
      (*b*) M. pharyngis siccus. Colonies dry and tough and adhere to medium.

NOTE.—Other biscuit-shaped Gram-negative organisms resembling the Meningococcus are (*a*) *Diplococcus flavus*. The colonies show yellow pigment and we have three varieties according to the depth of the yellow color. (*b*) *M. pharyngis siccus*, with yellowish, dry tenacious colonies and (*c*) *M. cinereus* with coarse dry colonies on ascitic agar. Like *M. catarrhalis*, it does not ferment any of the above-mentioned sugars. The individual cocci, however, are larger and more oblong in shape. A very important differentiating point is that these meningococcus-like organisms, with the exception of *M. catarrhalis*, agglutinate in 1–50 normal horse serum while true meningococci only agglutinate in immune horse serum.

STREPTOCOCCUS FORMS

Those cocci tending to arrange themselves in chains are usually described as streptococci. (Ogston, 1881; Rosenbach, 1884.)

When we consider that certain bacilli at times assume an arrangement which we term streptobacilli, yet have no relationship, it would suggest that the matter of chain morphology is simply a characteristic common to many entirely different cocci.

Again old laboratory cultures of streptococci may show alternations of cocci and rods giving the appearance of the dots and dashes of the Morse code. Furthermore unsuitable media may bring about various involution types in an organism primarily streptococcal.

It is often difficult to distinguish streptobacilli from streptococci morphologically and the same is true of diplococci and diplobacilli. These bacillary pairs and chains, however, often show bipolar staining and are almost invariably Gram-negative.

While streptococci tend to assume chain formation in pus and tissues they often appear as diplococci in blood.

The essential point to bear in mind is that the finding of a streptococcus does not necessarily explain an infection, because normally streptococci are among the organisms most frequently and abundantly found in plates made from normal buccal and nasal secretions. It is well to be very conservative when reporting streptococci as the etiological factor from lesions of the throat or nose.

FIG. 13.—*Streptococcus pyogenes.* (*Kolle and Wassermann.*)

Probably the most practical point in the differentiation of streptococci, next to that of pathogenicity, is the occurrence of long or short chains, the virulent ones tending to appear in chains of from 10 to 20 cocci, while the normal inhabitants of the nose, mouth and fæces generally tend to be in shorter chains.

Virulence.—As regards virulence, this is exceedingly variable—it is soon lost, but may be restored either by inoculating streptococci along with various other organisms or by passage through successive rabbits. The rabbit is the most susceptible animal and should be inoculated in one of the prominent ear veins. If the needle of the syringe is not inserted in the vein it will be difficult to force in the material and a swelling will immediately show itself.

Recently isolated cultures from human infections are not very virulent for animals. Passage through the rabbit, however, enormously increases the virulence. It

may be more convenient to inoculate a mouse at the root of the tail. If the culture is very virulent, it becomes generalized and death occurs in two or three days. If less virulent, a local abscess forms.

Hæmolysis.—Besides the morphological and pathogenic variations, Schottmuller has noted differences where these organisms are grown on 1 part of blood and 3 to 6 parts of agar. On this medium *Strep. erysipelatis* has a hemolytic action, the laking of the red cells bringing about a more or less clear ring surrounding the colony. This organism is often termed *S. hæmolyticus*. It tends to produce suppurative arthritis in rabbits while *S. viridans* causes endocarditis and the *Pneumococcus* an acute sepsis.

The hæmolytic substance is much like a ferment and is found in filtrates of cultures. The short-chain streptococci do not have a hæmolytic halo. They also have a greenish appearance like the *Pneumococcus* (*S. viridans*). The *Pneumococcus* has a greenish zone. Streptococci which are profoundly toxic and which have been isolated from milk-borne epidemic sore throats differ from the ordinary *S. pyogenes* in being encapsulated, not tending to form chains and producing only slight hæmolysis on blood agar.

Carbohydrate Reactions.—Some of the English authorities have introduced biochemical methods of differentiating: the *Strep. pyogenes* coagulating milk, reducing neutral red, and producing acid in lactose, saccharose and mannite media.

Practically all streptococci ferment glucose and saccharose. Blake separates streptococci first by effect of colony on blood agar. The methemoglobin and indifferent colonies (green ones or without color appearance) are separated by effect on mannite and lactose as follows:

S. buccalis = Lactose+, Mannite−.
S. fecalis = Lactose+, Mannite+.
S. equinus = Lactose−, Mannite−.

S. pyogenes does not produce acid in inulin media while the *Pneumococcus* does.

The most important point differentiating streptococci from pneumococci is the bile solubility of the pneumococci. Of streptococci not fermenting inulin we have *S. pyogenes*, with its hæmolytic zone around the colony; *S. viridans*, with its green colony, and *S. fæcalis*, which gives colonies neither distinctly green nor hæmolytic.

A freshly prepared solution of sodium taurocholate, 5%, added to an equal amount of a twenty-four-hour bouillon culture of *S. pyogenes* does not disintegrate the cocci or, at any rate, not within a few minutes. The reverse is true of the *Pneumococcus*. Formerly we recognized organisms showing marked capsule formation and mucilaginous growth as streptococci. As a rule these organisms are pneumococci belonging to group III (*Pneumococcus mucosus*). At the same time we do recognize a *Streptococcus mucosus* which is not soluble in bile, does not ferment inulin,

is not pathogenic for mice and has rather a tendency to produce slight hæmolysis on blood agar.

When we consider the biochemical variations which a single organism, as the colon bacillus, may exhibit, the value of such methods of differentiating may well be questioned. The question of the symbiotic relationship, which, when established between two or more bacteria, may cause harmless organisms to take on virulence, would appear to be a more important consideration.

Almost without exception, human streptococci are Gram-positive. Their colonies are quite small but distinct and discrete. In appearance the colonies of streptococci and pneumococci are practically identical. In a blood-serum throat culture *Pneumococcus* and *Streptococcus* colonies are the smallest, diphtheria ones are quite small and discrete, but slightly flatter. (Always examine the water of condensation for streptococci.) The *Sarcina* and *Staphylococcus* colonies are much larger.

Streptococcic colonies on blood agar are much more moist and luxuriant than on ordinary agar. A very important point, in judging whether a *Streptococcus* or other organism is pathogenic in a given infection, is to examine smears from the pus or other material in a Gram-stained specimen for information as to abundance and, in particular, phagocytosis of any organism, before plating out.

Pathogenic Effect.—Streptococci are commonly the cause of diffuse phlegmonous inflammations, while the staphylococci cause circumscribed lesions. Streptococci cause necrosis and do not characteristically produce pus. The importance of the *Streptococcus* as a secondary infection in diphtheria, tuberculosis, small-pox, and even in typhoid fever must always be kept in mind. It is this infection which does not respond to diphtheria antitoxin, and not the diphtheria one.

Rosenow has reported on the rather constant presence of streptococci in gastric and duodenal ulcers removed at operation, under which circumstance the number and variety of bacteria present are comparatively few. The strains from 27 chronic ulcers gave grayish-green colonies on blood plate, were in short chains and diplococci, produced much acid and turbidity in dextrose broth and showed a low-grade virulence. When injected into dogs, rabbits and guinea-pigs they showed a tendency to localize in the mucosa of stomach and duodenum, causing ulceration in a large percentage of cases. Rosenow believes poliomyelitis a streptococcal infection.

Streptococci as well as colon infections are always to be thought of in connection with cholecystitis and appendicitis.

It has been claimed that scarlet fever is a streptococcal infection (*S. anginosus*). Klimenko found streptococci only 11 times in the blood of 523 cases of scarlet fever. The Dohle inclusion bodies of the disease suggest chlamydozoal virus. Mallory has very recently claimed that scarlet fever is due to a diphtheria-like

bacillus. It is found in the same locations as the diphtheria organism and also produces a toxin which, however, is less virulent and only produces inconspicuous lesions. The membrane formation in the throat in scarlet fever is due to streptococci.

When freshly isolated from human lesions streptococci often show only a slight virulence for animals. Hence massive doses are indicated and intravenous or intraperitoneal injections. The guinea-pig is not very susceptible to streptococci; the rabbit and white mouse being the animals of choice.

In nondiphtheritic anginas, puerperal fever, ulcerative endocarditis and coccal enteritis it is the *Streptococcus* which is usually the cause. It has been claimed that acute articular rheumatism is due to a short-chain streptococcus (*M. rheumaticus*), which is best isolated from material from an acute joint infection, but may also be isolated occasionally from the blood. It produces much acid and clots milk in two days. The growth is described as being more luxuriant than that of *S. pyogenes*. It is about 0.5μ in diameter.

In the very common and fatal measles, bronchopneumonias and empyemas of service camps *S. hæmolyticus* has almost constantly been found as cause. The importance of carriers of this organism cannot be overestimated in measles wards. Cole found 11.4% of measles cases carrying *S. hæmolyticus* on admission, 38.6% after 4 days and 56.8% after 8 to 16 days. Prophylactic vaccination may be indicated. Recent work would indicate that *S. hæmolyticus* not infrequently causes lobar pneumonia. The same is true of mastoiditis.

Of the nonpathogenic streptococci the most important one is *S. lacticus*, which is described under milk. This differs from *S. pyogenes* in growing at lower temperatures and having greater viability. It is a normal inhabitant of cows' dung.

Serum Therapy.—Antistreptococcic serum is antimicrobic. There is some indication that this serum, when made with strain of *S. hæmolyticus*, has possible value, but the antiserum for *S. viridans* seems to have no effect.

Sarcina Forms

These are best observed in hanging-drop preparations, when they can be seen as little cubes, like a parcel tied with a string, and by noting them when turning over, it will be seen that they are different from the tetrads which only divide in two directions of space. At times the packet formation is not perfect and it will be difficult to distinguish such as sarcinæ. All sarcinæ stain by Gram. If the staining of sarcinæ be too deep it may obscure the lines of cleavage. Sarcinæ are nonmotile.

Various sarcinæ have been isolated from the stomach, especially when there is stagnation of stomach contents. Sarcinæ have also been found in the intestines. In plates the *S. lutea* is frequently a contaminating organism, being rather constantly present in the air. The demonstration of sarcina morphology should always be made from liquid media, as bouillon. Urine makes an excellent medium.

Micrococcus Forms

This grouping includes all cocci which do not show chain or packet formation. It will be found convenient to divide them into two classes according to their staining by Gram. The *M. tetragenus*, *S. pyogenes aureus* and the *Pneumococcus* stain by Gram, while the *Gonococcus*, the *Meningococcus*, the *M. catarrhalis* and the *M. melitensis* are Gram-negative.

M. Tetragenus.—This organism is frequently found associated with other organisms in sputum, especially with tubercle and influenza bacilli. The colonies are white, slightly smaller than staphylococci and are quite viscid.

It was formerly considered unimportant in disease, but the idea now prevails that it is responsible for many abscesses about the mouth, especially in connection with the teeth. Injected subcutaneously into Japanese mice, it produces a septicæmia and death in three or four days. The blood shows great numbers of encapsulated tetrads. It has been reported twice as a cause of septicæmia in man.

Staphylococci.—To cocci dividing irregularly and usually forming masses which are likened to clusters of grapes the term *Staphylococcus* is applied. While there have been experiments which show that by selecting pale portions of a yellow colony, eventually a white colony could be produced, yet, as a practical consideration, it is convenient to consider at least two types of staphylococci: the *Staphylococcus pyogenes aureus* and the *Staphylococcus pyogenes albus*. In culturing from the pus of an abscess or furuncle we generally obtain a golden coccus, while in material from the nose or mouth, the *Staphylococcus* colonies are almost invariably white. As regards the common skin coccus, this will be found to produce a white colony. A coccus which very slowly liquefies gelatin and has been supposed to cause stitch abscesses is the *S. epidermidis albus*.

While it is customary to look for a golden colony in the case of organisms showing virulence, yet at times a cream-white colony may develop from cocci of great virulence. Staphylococci show marked resistance to dessication and dried pus may contain live organisms for months. Old bouillon cultures of staphylococci contain a

ferment-like substance, leucocidin, which disintegrates leukocytes. Such cultures may also show a hæmolysin and when filtered and injected into animals show destructive action on cells of various organs. Amyloid change may be caused in animals by repeated injections of either living or dead cultures.

The *S. pyogenes citreus* is considered as of very feeble pathogenic power. Certain cocci whose colonies have presented a waxy appearance have been designated as *S. cereus albus* and *S. cereus flavus*, respectively. They are of very little practical importance. The *Staphylococcus pyogenes aureus* grows readily at room temperature, but better at 37°C. It coagulates milk and renders bouillon uniformly turbid. It grows on all media, as blood-serum, agar, potato, etc. It has been proposed to distinguish it from skin staphylococci by its power of producing acid in mannite. Ordinarily the individual cocci are about 1μ in diameter, but they vary greatly in size according to the age of the culture and other conditions. The "aureus," as it is frequently called, is not only often found in circumscribed processes, but it is a frequent cause of septicæmia, osteomyelitis, endocarditis, etc. In the tropics staphylococcal infections often show great virulence and clinically may resemble streptococcal ones. Smears from such erysipelatoid lesions show diplococcal morphology, often phagocytized. A pemphigoid eruption in children is often staphylococcal (Pyosis).

FIG. 14.—Gelatine culture *Staphylococcus aureus* one week old. (*Mac Neal.*)

In infection of bone tissue the *Staphylococcus* is by far the most frequent cause. It is well to remember that insignificant staphylococcal infection may lead to septicæmia. In the tropics, where resistance is often lowered and staphylococcal skin infections common, continued fevers are often septicæmias. It is the organism most frequently concerned in terminal infections. The lowered resistance of the patient permits of their passage through barriers ordinarily resistant. Not only should this be kept in mind when such organisms are isolated at an autopsy, but as well the fact that their entrance may have been agonal or subsequent to death. Vaccines have been most successful in the treatment of staphylococcal infections. They stimulate phagocytosis. Pyelitis may be due to staphylococci.

The Pneumococcus of Fraenkel.—(Pasteur and Sternberg in 1880. Fraenkel, 1884, isolated it from normal persons as well as pneumonia patients. Inoculated mice and rabbits. Hence Fraenkel's organism. Weichselbaum accurately differentiated organisms causing pneumonia in 1886.) This is by far the most common cause of lobar pneumonia. It is also frequently found in meningitis, empyema, endocarditis and

otitis media. It should not be confused with the pneumobacillus of Friedländer, which, although possessing a capsule like the *Pneumococcus*, differs from it by being Gram-negative, being a bacillus and having large viscid colonies. The *Pneumococcus* is the cause of more than 80% of the cases of lobar pneumonia.

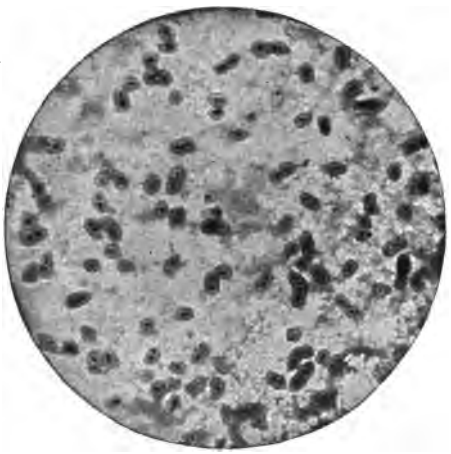


FIG. 15.—*Pneumococcus*, showing capsule, from pleuritic fluid of infected rabbit, stained by second method of Hiss. (*Mac Neal.*)

In 529 cases the Rockefeller Institute reports give the following causation table:

Diplococcus pneumoniæ	454
Friedländer's bacillus	3
Bacillus influenzæ	6
Streptococcus pyogenes	7
Streptococcus mucosus	1
Staphylococcus aureus	3
Cases of mixed infection with combinations of *Staphylococcus aureus*, Friedländer's bacillus, *B. influenzæ*, *Streptococcus pyogenes*, and *Streptococcus viridans*	6
Undetermined (most of them occurring before accurate methods for determining the etiologic agent had been devised)	49

It does not grow below 20°C. and is best cultivated on blood-serum, or blood agar. On plain agar it grows as a very small dew-drop-like colony, which is slightly grayish by reflected light. It produces considerable acid, thus acidifying and usually coagulating litmus milk. It produces acid in inulin media which the *Streptococcus* fails to do. The most important differentiating point is its bile solubility. The colony is smaller and more transparent than a *Streptococcus* colony.

In sputum or other pathological material it is best recognized by the presence of a capsule inclosed in which are two lance-shaped cocci with their bases apposed. In artificial culture we rarely get the capsule. It also sometimes grows in short chains like a *Streptococcus*. The best medium for differentiating is the serum of a young rabbit; in this it grows as a diplococcus, while streptococci show chains. Rosenow, by combining passage in animals with culturing symbiotically with *B. subtilis* claims to have changed the *Pneumococcus* into a hæmolytic *Streptococcus*. The best method of isolating it in pure culture is to inject the sputum into the marginal ear vein of a rabbit or subcutaneously into a mouse. Death results from septicæmia in about two days and the blood teems with pneumococci. Usually the *Pneumococcus* quickly loses its virulence, and also dies out in a few days unless transferred to fresh media. Virulence is quite variable and is attenuated by subculturing and exalted by animal passage. Usually the more toxic a case of pneumonia the more virulent the pneumococci, so that 0.000001 c.c. of a broth culture may kill a mouse in thirty-six hours. The best medium for its preservation is rabbit's blood agar; this also maintains the virulence. On this medium the colonies are larger than on agar and they present a greenish appearance. They are flat, ringed and surrounded by a greenish zone of methæmoglobin.

The *Pneumococcus* growth emulsifies very readily and evenly so that suspensions for vaccines are easily made.

It is a well-known fact that *Pneumococcus* is a frequent inhabitant of the nasal, pharyngeal, and buccal cavities. The explanation of infection is either on the ground of lowered resistance of the patient or enhanced virulence of the organism. Vaccines appear to be valueless in pneumonia but may be useful in local infections. They probably stimulate opsonin production.

In a study of blood and sputum cultures from 32 cases of lobar pneumonia Hastings and Boehm found blood and sputum positive bacteriologically in 11 cases. In 9 of these cases the *Pneumococcus* was isolated and in 2 a hæmolysing *Streptococcus*. In the other 21 cases the sputum cultures were bacteriologically positive in 18 of the cases and negative in 3. In 9 cases the *Pneumococcus* was isolated, in 2 cases *B. coli*, in 1 case *M. catarrhalis*, in 1 case a *Staphylococcus*, in 2 cases staphylococci and streptococci, in 1 case *B. influenzæ*. The percentage of positive blood cultures was 30.3. Cole obtained 30% of positive blood cultures. The blood was taken into flasks of bouillon in dilution of 1–50. In the cases giving positive blood cultures the mortality was 55%. In the negative ones 8%.

Types of Pneumococci.—Neufeld showed that different strains of pneumococci differed immunologically. Cole and his colleagues at the Rockefeller Institute Hospital have elaborated this work and divided pneumococci into four groups. Of these, organisms belonging to group III can be recognized in a Gram stained preparation by their large capsules and viscid colonies, the groups I and II can be differentiated by serum reactions and to group IV belong pneumococci which cannot be placed in the other groups. The common name for group III pneumococci is *P. mucosus*.

In cases of pneumonia group I was found in 33%, group II in 31%, group III in 12% and group IV in 20%. The mortality in such cases was 23, 32, 45 and 16% respectively.

Types I and II are the strains most often present in epidemics of pneumonia and have been referred to as "epidemic strains." They are rarely found in the throats of those who have not been in contact with cases; the normal throat types usually belonging to group IV.

In a study of the saliva of 297 non-contacts, group I organisms were only found once and group II ones not at all. Contacts with type I cases gave 13% and with type II 12% of positives. These contact carriers usually harbored the organisms for only three or four weeks. Even cases of the disease usually cease to show these epidemic types in their sputum within 3 or 4 weeks. For methods of differentiating the groups see under agglutinins and precipitins.

Serum Treatment.—An efficient therapeutic serum has been prepared against type I organisms but not for other groups. The serum has no beneficial effect on cases due to other types. The serum is prepared by injecting horses first with dead organisms and subsequently with living ones. It is necessary to use a highly virulent strain and the organisms are injected intravenously. A satisfactory serum in amounts of 0.2 c.c. should protect a mouse against 0.1 c.c. of a culture whose fatal dose is 0.000001 c.c.

The serum is given intravenously in repeated doses of 50 to 100 c.c. The average amount of serum required for a case is about 250 c.c. Before giving the serum carry out intradermal and desensitizing tests. See Anaphylaxis.

Diplococcus Crassus.—This is a Gram-positive, kidney-shaped diplococcus, which might be confused with the *M. catarrhalis* or the *Meningococcus* by ordinary staining methods. It is larger than the *Meningococcus*.

It is not strongly Gram-positive as one may find examples in the same preparation about which doubt may be entertained. It ferments lactose and saccharose as well as glucose and maltose. It agglutinates with normal horse serum.

In throat cultures I have isolated on several occasions a Gram-positive diplococcus which is at times biscuit-shaped, at times irregularly spherical. It possesses two or three metachromatic granules, so that in a Neisser stain for diphtheria the appearance of these granules may be confusing.

Using Ponder's toluidin blue stain I have observed granule staining in organisms of round or oval morphology which were suggestive of the ascospore staining of yeasts. Staphylococci may show granules with Ponder's stain.

Gram-negative Cocci.—It is important to bear in mind that there are many cocci of varying shapes, which in cultures or in smears from the throat, nose or fæces are Gram-negative. These are not well classified or described. To distinguish the three important kidney-shaped diplococci, it can be most easily accomplished by cultural methods,

using hydrocele agar (ascites or blood agar will answer), ordinary blood-serum and plain agar. The *Gonococcus* will only grow on the hydrocele agar; the *Meningococcus* will grow on this, but likewise grows on ordinary blood-serum. The *M. catarrhalis* will grow on plain agar as well as on other media.

Other Gram-negative organisms of confusing morphology are *M. pharyngis siccus*, the colonies, of which show great crinkly dryness, and *M. pharyngis flavus*.

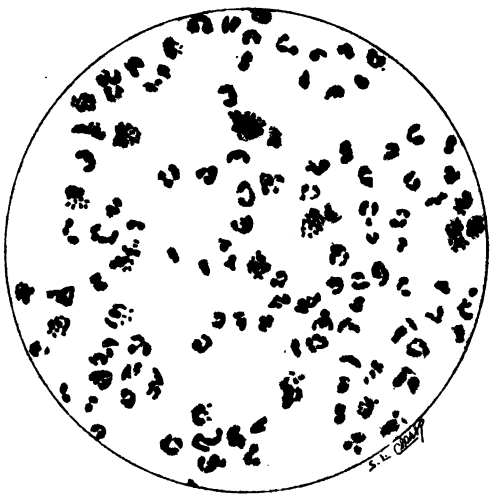


FIG. 16.—*Gonococcus*. Film from urethral pus. (*Coplin*.)

Gonococcus (Neisser, 1879).—This organism is characteristically a diplococcus, the separate cocci being plano-convex with their plane surfaces apposed. (Biscuit shape, coffee-bean shape.) They are generally found grouped in masses of several pairs, most strikingly in pus cells or epithelial cells, but also found extracellularly. Except in the height of the disease, there is a great tendency for the organisms to show involution forms, so that instead of biscuit-shaped diplococci we have round, irregular and uneven cocci.

It is therefore advisable in searching smears from chronic gonorrhœa to continue the search of Gram-stained specimens until some fairly typical diplococci are found. There is nothing requiring greater discrimination than a diagnosis from such a smear. At the commencement of a gonorrhœa the epithelial cells are abundant and gonococci are found adhering to them or lying free. Later on, at the acme of the discharge (the creamy, abundant discharge), it is in the pus cells we find them

and they may be so abundant that 10 to 20% of the pus cells may contain them. In the subacute stage the epithelial cells, which practically disappear when the discharge is so abundant, begin to reappear, and in the chronic stage the epithelial cells are the chief ones, and are the ones on which we find an occasional gonococcus, often distorted in shape. In gonorrhœal ophthalmia the gonococci may show appearances in the conjunctival epithelium resembling the inclusion bodies of trachoma.

The best method of diagnosis in cases of chronic gonorrhœa is to have the patient eat the stimulating food previously interdicted, to take active exercise and to have a sound passed. To obtain material for examination the glans penis should be washed and the patient who has presented himself with a full bladder should pass a portion of the contained urine. Next the prostate and seminal vesicles should be massaged with the patient standing but bent over and the penis pendant. The drops of discharge from the massage should be received in a small Petri dish and finally the remaining urine should be passed into a sterile bottle. Smears and cultures should be made from the sediment of the two urinary specimens and from the secretions of the massaged prostate and vesicles.

The smears made from the resulting discharge or centrifuged urine will probably contain gonococci if they are present in the urethra. In the female the favorite sites are the urethra and the cervix uteri. In municipal examinations it is customary to make two smears: one from the urethral meatus and a second from the cervix. The vagina is not a suitable soil for their development. In female children it is most often found in the discharge of the vulvovaginitis. Gram-stained smears from pus sediments of urine, especially in pyelitis or cystitis, may show coccoid forms of *B. coli*, which may be phagocytized and thus be reported as gonococci.

In addition to the genital organs, the *Gonococcus* may at times invade and be isolated from the eye (gonorrhœal ophthalmia), the joints, rarely as a cause of endocarditis and possibly as the factor in septicæmia. Grown upon hydrocele or ascites agar, or blood-streaked agar, or upon blood agar from man or the rabbit, the colonies appear as irregular, minute, dew-drop spots. By the second or third day the involution forms are abundant, and within four to seven days the culture will probably be found to be dead. Unless frequent transfers are made, it will be best kept alive on blood agar. The organism grows best at 37°C., and will not grow below 25°C. It will not grow on plain or glycerine agar or ordinary blood-serum unless the transfer of considerable pus in inoculating the slants gives it a suitable culture medium. In material from joints, it is in the fibrin flakes that the gonococci are most apt to be found, if found at all.

Animals do not contract gonorrhœa. Even in monkeys urethral inoculations of gonococci are negative. The organism is killed in five hours by a temperature of 45°C. and speedily by drying. In moist smears of pus it may live for one or two days.

By heating the blood-streaked agar tubes at 56°C. for twenty minutes (inactivation-destroying complement and hence bactericidal power of blood on slant) greater success in primary cultures will be obtained.

In culturing gonococci the transfer of material to culture media should be made with the least delay possible.

The most satisfactory medium is Thalman's medium upon the slanting surface of which we have deposited two or three drops of human serum. Blood may be taken from a vein or the Wright U tube may be used and after centrifuging the sterile serum is taken off with a capillary bulb pipette and deposited on and smeared out on the slant.

Diplococcus Intracellularis Meningitidis (Weichselbaum, 1887).—This is the organism of epidemic cerebrospinal meningitis, and is frequently termed the *Meningococcus*. The diplococcus is Gram-negative and biscuit-shaped and is, like the *Gonococcus*, chiefly contained in pus cells. It is also found free in the cerebrospinal fluid withdrawn from cerebrospinal fever cases. There is a greater tendency to variation in size and shape than is the case with the *Gonococcus*, which latter, in fresh material, shows a striking uniformity morphologically. The *Meningococcus* is at times not abundant. Early in the case, however, the picture may be similar to that of gonorrhœal smears.

At times we may find the meningococci microscopically but not upon culturing the spinal fluid or, we may obtain positive cultures where organisms were not found in the smear.

Rarely it is impossible to demonstrate the presence of meningococci, especially where the case may show a fairly clear fluid (with only slight flocculent material) instead of the turbid one usually withdrawn.

On blood-serum the colonies appear after twenty-four to forty-eight hours as discrete, very slightly hazy colonies, about $\frac{1}{10}$ inch in diameter. On serum agar, as ascites or hydrocele agar, they grow best and show as faint bluish colonies about 1 to 2 mm. in diameter. They are larger than *Streptococcus* or *Pneumococcus* colonies. Unless considerable cerebrospinal fluid is transferred with the inoculating loop, they do not grow on plain agar. They will grow at times on glycerine agar. The best medium for plate cultures with carriers is blood agar. The hæmolytic and green appearing colonies of streptococci and the greenish pneumococcus colony are readily differentiated. The meningococcus colony is slightly opaque in the center and rather clear at the periphery. While laked blood agar plates have the advantage of a lens effect with transmitted light yet on the whole the more opaque blood agar is to be preferred. The organism is very sensitive to light, cold and drying. It ferments glucose and maltose but not lactose or saccharose and only grows at blood temperature, thus distinguishing it from the *M. catarrhalis* which will not ferment any of these sugars, and grows at room temperature. It is scarcely pathogenic for laboratory animals, with the exception of the mouse and guinea-pig, when intraperitoneal injections but not subcutaneous ones give results. Intradural injections give results. The cultures die out very rapidly, so that it is

necessary to make transfers every one or two days. Stock cultures are best kept on blood agar slants. The *Meningococcus* has been isolated from the nasal secretions of patients but on the whole nasal cultures are negative, the organisms preferring the posterior naso-pharynx. The possibility of these organisms being the *M. catarrhalis* must be considered. Of the greatest importance is the examination of the naso-pharyngeal material of those who have been in contact with a patient. These healthy carriers are important. To examine such people introduce a bent wire applicator with sterile cotton tip past the soft palate so as to get material from the naso-pharynx. The material should be immediately inoculated on blood or serum agar and quickly put in the 37°C. incubator. The plates should be warm when inoculated and kept at 37°C. until placed in the incubator.

Flexner has shown that in monkeys, which are susceptible to the disease, injections of cultures of *M. intracellularis* into the spinal canal is followed by migration of the cocci to the nasal cavity both free and in phagocytic leukocytes.

The *Meningococcus* has a very slight resistance to sun or drying so that its aërial transmission seems doubtful. It is supposed to effect an entrance by the nares, thence reaching the cerebral meninges. At present our views are that meningitis is a blood infection at first and later the meningococci locate in the central nervous system. We now make blood cultures as well as spinal fluid ones. We also now inject serum intravenously as well as intrathecally. Infection is probably by direct contagion. Several cases have been reported where with a high leukocytosis the cocci have been found in the polymorphonuclears of blood smears and in cultures from the blood. (In about 25% of blood cultures where from 5 to 10 c.c. are employed.)

Serum Treatment.—By the use of initial injections into horses of killed cultures followed by alternate injections into horses of living diplococci, then seven days later of an autolysate made from different strains; seven days later again injecting living diplococci; thus alternating material every week, an antiserum of value has been obtained by Flexner. The immunization requires about one year. More recently it has been possible to greatly shorten this period. Withdraw spinal fluid until pressure is relieved and fluid comes out by drops (4 or 5 per minute). Leaving needle in place inject serum in amount equal to about ½ spinal fluid removed. Repeat injection according to symptoms. Intravenously use 50 to 100 c.c.

Types of Meningococci.—Dopter first called attention to the existence of two types which differed immunologically—the normal and the para strains. The Rockefeller Institute recognizes these two types and also irregular types. The English recognize 4 types—I, II, III and IV. Type I corresponds to Flexner's para strain and type II to his normal one. Types III and IV are irregular ones. In treatment we always use a polyvalent serum made from as many strains or types as may show differences in immunity reactions.

Gordon notes that there is somewhat close affinity between his types I and III and between types II and IV. He also notes that chronic carriers tend to continue to carry the same type organism. As to frequency of types in cases in England he has found the following: For type I 40%, type II 45%, type III 10 and type IV 5%. The types found in cases and in carriers show a close correspondence.

For diagnosis, make smears and cultures from cerebrospinal fluid. The sediment from the centrifuged material gives better results. In tuberculosis the lymphocytes preponderate; in cerebrospinal meningitis the polymorphonuclears. For final diagnosis of meningococci, as well as for type, agglutination reactions are necessary. See Agglutinins.

FIG. 17.—*Diplococcus intracellularis meningitidis* and pus cells. (× 1000.) (*Williams.*)

It has been stated that a point of difference between the phagocytosis with the gonococci and the meningococci is that the meningococci invade and at times destroy the nucleus of the polymorphonuclear, which is not true of gonococci. The appearance of large phagocytic endothelial cells, often containing polymorphonuclears, in the centrifuged cerebrospinal fluid is a favorable prognostic sign. At times there does not appear to be any relation between the number of phagocytic polymorphonuclears and the severity of the case.

Carriers.—In the examination of incoming recruits for the military services it is not unusual to find as many as 2% showing meningococci when their throats are cultured. Those in contact with cases of meningitis or well carriers may show a high incidence—10%, or even higher.

Most cases cease to be carriers within a few weeks and well carriers tend to follow the same rule. It is a question whether treatment of carriers is of much avail. Warm weather, especially when combined with tent life tends to cause the

organisms to disappear from the naso-pharynx. There are some cases which persist in the carrier state in spite of any treatment or environment. These "persistent" carriers may give an occasional negative culture, but if we demand 3 or 4 successive negative cultures, at intervals of five days, before pronouncing the man free from his infection the carrier period may be prolonged for months.

It is striking the rarity with which such carriers come down with the disease, but resistance reducing factors, as colds, excessive fatigue, chilling, etc. may cause them to develop the disease.

The English naval statistics would indicate that their cases almost always develop in those who have previously shown negative cultures. American statistics fail to show the same. Young children are most susceptible to meningitis and contact on their part with a carrier is more dangerous than for an adult. Carriers are vastly more common in winter than summer and the disease is peculiarly a disease of cold, inclement weather.

Micrococcus Catarrhalis (Seifert, 1890).—This organism has been specially studied by Lord. It resembles the *Meningococcus* strikingly and can only be differentiated by cultural procedures. It grows on plain agar and at room temperature, and does not produce acid in glucose media. It not only occurs in the nasal secretions of healthy people, but appears to be responsible for certain coryzas and bronchial affections, resembling influenza. It also is responsible for certain epidemics of conjunctivitis (chiefly differentiated from *Meningococcus* by agglutination).

The original cultures may show only slight growth whereas the subcultures prove luxuriant.

The colonies are larger, more opaque, and have a more irregular wavy border than the round colonies of the *Meningococcus*.

The colony tends to be easilypicked up from the plate with the loop. *M. catarrhalis* grows well at 22°C. after several days, while the *Meningococcus* requires body temperature. It does not ferment with acid production any of the sugars.

Micrococcus Melitensis (Bruce, 1887).—This is the organism of Malta or Mediterranean fever, sometimes called undulant fever, on account of successive waves of pyrexia running over several months. The disease has a very slight mortality (2%), and the lesions are chiefly of the spleen, which is large and diffluent. The organisms can best be solated from the spleen or blood.

M. melitensis is only about 0.3μ in diameter. The characteristics are its very small size and the dew-drop minute colonies on agar, which at incubator temperature only show themselves about the third to the sixth day. It is nonmotile and Gram-negative. In bouillon there is a slight turbidity. Gelatine growth is very slow and

there is no liquefaction. Litmus milk becomes more blue after a week so that there is an alkaline action. Indol is not produced. The optimum reaction of media is + 0.8. It grows best at 38°C.

Many laboratory infections have been recorded.

The organism occurs in peripheral circulation, it having been cultivated from blood very successfully by Eyre. He takes blood at the height of the fever, and in the afternoon. Formerly it was customary to isolate by splenic puncture.

Infection is chiefly by means of the milk of infected goats. The organisms are excreted in the urine of patients, and a diagnostic point is to make plates from the urine. Such urine applied to abraded surfaces causes infection.

The serum of patients shows agglutinating power as early as the fifth day of the disease, and this may persist for years after recovery. Nicolle has advised using serum heated to 56°C. for thirty minutes for the agglutination test, nonspecific agglutinins being thereby destroyed. Carriers may be of importance in Malta fever and are best detected by agglutination tests.

A high mononuclear increase may be found in this disease.

Horses, cows, asses, as well as goats, are susceptible. It is very difficult to infect rabbits, mice and guinea-pigs. Monkeys have been chiefly utilized in experimental work.

It would appear as if there were other organisms closely related to *M. melitensis* and a great deal is now being written as to confusing serum reaction from the use of *M. paramelitensis*.

What may be deemed proof positive of goats' milk transmission is the practical disappearance of the disease among the naval and military forces of Malta, as the result of boiling the milk, while still continuing among native civilians not boiling their milk. Bassett-Smith has noted that in 1905 there were 798 cases among civilians and 245 naval cases. In 1907 there were 457 cases among civilians and only twelve cases in the naval forces.

There are however occasional cases which Shaw has considered as due to carriers. As the organisms are excreted in fæces as well as in urine, and as the course of the disease is so protracted, as well as the convalescence, it would seem that the carrier factor should be of more importance than facts would justify.

Mohler has noted in Texas, where the disease has existed for twenty-five years, that the Mexican goatherds boiled their milk and hence were rarely infected.

The souring of milk does not destroy the germs of the disease, hence transmission may be brought about by butter and cheese.

Malta fever was stamped out of Port Said by destroying all infected goats.

Infection may occur, 1. by the stomach atrium (usual), 2. contaminated dust reaching lungs, 3. by subcutaneous infection.

CHAPTER VI

STUDY AND IDENTIFICATION OF BACTERIA. SPORE-BEARING BACILLI. KEY AND NOTES

A. **Grow aerobically.**

1. Stab culture in gelatin has branches growing out at right angles to line of stab.
   (*a*) Has no membrane on bouillon or liquefied gelatin. Projecting branches from line of stab only at upper part of line of growth. Absolutely non-motile. Ends sharply cut across or concave. ANTHRAX GROUP.
   (*b*) Has thick whitish membrane on bouillon and surface of liquefied gelatin. Projecting branches all along the line of stab. Sluggishly motile. MYCOIDES GROUP. (B. mycoides. B. ramosus.)
2. Stab cultures in gelatin do not show projecting branches.
   (*a*) Potato cultures do not become wrinkled. At first slightly moist, later dry and mealy. SUBTILIS GROUP. (Hay bacillus group.) Actively motile with more or less square ends and a central spore which is of the same diameter or only slightly larger than the bacillus. The yellow subtilis is at times found in water. The colonies on potato are of a cheese-yellow color. The bacilli are very large and show a sluggish, worm-like motion.

   B. megatherium often shows a granular or beaded appearance in a Gram preparation. The narrow spores are never central, usually between center and end, and rather elongated. It most nearly resembles the sporulating bacillus of malignant œdema but if the spore is quite terminal and bulging may resemble B. tetani.

   Cultures of B. megatherium are somewhat similar to B. coli colonies.
   (*b*) Potato cultures at first even growth but after a few days become wrinkled. VULGATUS GROUP. (Potato bacillus.)

   B. vulgatus shows marked wrinkling, like intestinal coils. B. mesentericus show slight wrinkling and a network-like appearance.

   Two water bacilli belonging to this group are the B. mesentericus fuscus (brown growth) and B. mesentericus ruber (red growth).

NOTE.—The following cultural characteristics are common to all the above spore bearers.

1. Liquefaction of gelatin.
2. Milk slowly and incompletely coagulated with very little change in reaction. Later the coagulum is digested.
3. No gas in either glucose or lactose.
4. No indol.
5. All are Gram-positive.
6. All digest blood-serum.

NONPATHOGENIC SPORE-BEARING AEROBES ON AGAR

Modification of table of Gruner and Fraser.

Surface dry	Gray white	B. subtilis (motile).
		B. ellenbachensis (non-motile).
	Chalk like	B. cretaceus.
	Edges feathery	B. mycoides.
Surface gummy	Yellow	B. ochraceus.
	White	B. mesentericus.
Surface moist and exuberant	Yellow	B. ruminatus.
	Dirty gray	B. graveolens.
	White and wrinkled.	B. vulgatus.

NOTES.—B. subtilis has square ends and central spores, not causing bulging.

B. megatherium has spore toward one pole which may be terminal.

B. vulgatus is long and slender. Slightly oval spores.

B. mesentericus varies. Usually short with rounded ends and central bulging spores.

B. ellenbachensis has rounded ends, oval spores and shows granule formation (beaded)—resembling diphtheroids.

All of these grow well at room temperature, optimum 30°C.

B. **Grow only anaerobically.**

1. Rods very little swollen by centrally situated spores.
   (*a*) Motile. B. œdematis maligni. (Gram-negative.)
   (*b*) Nonmotile. B. aerogenes capsulatus. (Capsule.)
2. Spores tend to be situated between center and end.
   (*a*) No liquefaction of gelatin. B. butyricus.
   (*b*) Gelatin liquefied slowly.
      B. botulinus. Milk not coagulated.
      B. anthracis symptomatici.
      B. enteritidis sporogenes. Milk coagulated with abundant gas.
   (*c*) Gelatin liquefied rapidly. B. cadaveris sporogenes. Very motile.
3. Spores situated at end of rod. Drum-stick sporulation. TETANUS GROUP.

The following table taken from Lehmann and Neumann, based on pathogenic effects, is of great practical value. After inoculation of some animal subcutaneously with the suspected material we have:

A. No particular symptoms at site of inoculation.
   Absorption of the soluble toxin causing:
   (1) General symptoms of tetanus. B. tetani.
   (2) Botulism poisoning symptoms. Pupillary symptoms. Paralysis of tongue and pharynx. Cardiac and respiratory failure.

B. Local symptoms marked at site of inoculation. Hemorrhagic emphysematous œdema.
   (1) Motile.
      (*a*) Gram-negative. B. œdematis maligni.
      (*b*) Gram-positive. B. anthracis symptomatici.

(2) Nonmotile.
B. aerogenes capsulatus, B. phlegmonis emphysematosæ (Fränkel) or B. perfringens.

Spore-bearing Aerobes

Bacillus Anthracis (Pollender discovered 1849. Davaine recognized nature 1863. Koch proved 1876).—Of the aerobic spore-bearing bacilli this is the only one of particular medical importance.

Anthrax is an important disease in domesticated animals, especially sheep and cattle. The characteristic postmortem change in animals is the greatly enlarged, friable, mushy spleen. Man is much less susceptible than these animals, but is more so than the goat, horse, or pig. The Algerian sheep has a high degree of immunity,

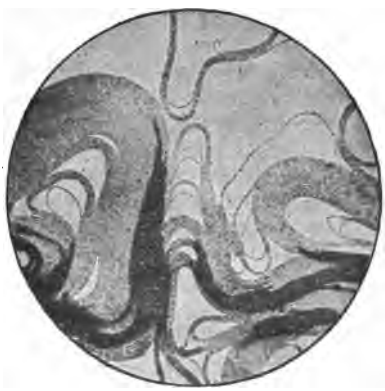


Fig. 18.—Anthrax bacilli. Cover-glass has been pressed on a colony and then fixed and stained. (*Kolle and Wassermann.*)

as has the white rat. The brown rat is quite susceptible as are also guinea-pigs, mice and rabbits. The disease in man chiefly occurs among those working with hides, wool, or meat of infected cattle. The two chief types in man are: 1. Malignant pustule and 2. Woolsorter's disease. An intestinal type is also recognized.

Malignant pustule results from the inoculation of an abrasion or cut; thus it frequently shows on the arms and the backs of those unloading hides. It first appears as a pimple, the center of which becomes vesicular, then necrotic.

A ring of vesicles surrounds this central eschar and a zone of congestion, the vesicles. The lymphatics soon become inflamed as well as neighboring glands. If the pustule is not excised and death occurs, there is not much enlargement of the

spleen and the bacteria are not abundant in the kidneys, etc., as with animals. Man seems to die from toxæmia rather than a septicæmia.

In woolsorter's disease there is great swelling and œdema of the bronchial and mediastinal glands. The lungs show œdema, which about the bronchi is hemorrhagic.

The bacillus is 5 to 8μ by 1 to 1½μ and nonmotile. In cultures it has square cut or concave ends and is often found in chains, but in the blood of an infected animal the free ends of the rods are somewhat rounded. It is Gram-positive. Colonies, by interlacing waves of strings of bacteria, show Medusa head appearance. For cultural char-

FIG. 19.—Anthrax bacilli growing in a chain and exhibiting spores. (*Kolle and Wassermann.*)

acteristics see key. Spores develop best at a temperature of 30°C. and do not form at temperatures above 43°C. They are oval and centrally placed. They stain with difficulty.

Stiles thinks that animals are infected by eating the bones of animals which have died of anthrax, cutting buccal mucous membrane, and so becoming infected. Spores do not form in an intact animal body, but they do form after a postmortem or the disintegration of the body by maggots. For this reason it is better not to open up the body of the animal, but to make the diagnosis by cutting off an ear. Dried spores will live for years and will withstand boiling temperature for hours.

In vaccinating animals against anthrax, Pasteur used two vaccines. The first is attenuated fifteen days at 42.5°C. The second, attenuated for only ten days, is given twelve days later.

Various bacteria, especially *B. pyocyaneus*, show marked antagonism to *B. anthracis*. Pyocyanase digests the anthrax bacillus and has been used to cure animals infected with anthrax.

In taking material from a malignant pustule before excision, be careful not to manipulate it roughly, as bacteria may enter the circulation. Make cover-glass preparations, staining by Gram. Make culture on agar. Blood cultures are usually only positive later in the disease. Inoculate a guinea-pig or a mouse subcutaneously.

The guinea-pig dies in about forty-eight hours and shows an œdematous gelatinous exudate at site of inoculation. The blood is black and swarms with anthrax bacilli. It is the best example of a septicæmia.

In cases where the infection becomes septicæmic instead of localized the outcome is usually fatal. In such cases one should inject anti-anthrax serum intravenously, in doses of 50 to 75 c.c.

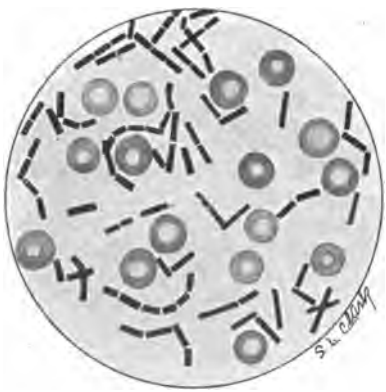


FIG. 20.—*Bacillus anthracis* in blood of rabbit. (*Coplin.*)

Symptomatic Anthrax.—There is an anaerobic spore bearer, called the bacillus of symptomatic anthrax, blackleg or quarter-evil, which causes a rapidly developing emphysematous swelling, with a dark color of the thighs. It is called *B. chauvoei.* It has bulging, slightly oval spores at one end, but they are not distinctly terminal as with tetanus spores. It affects sheep and cattle but not man. It is a soil organism like those of tetanus, malignant œdema and gas gangrene.

There is also an aerobic spore-bearing bacillus called *B. anthracoides*, which differs morphologically from anthrax solely in its rounded ends in culture. Its growth is more rapid and it liquefies gelatin more energetically.

SPORE-BEARING ANAEROBES

There are four very important pathogens in this group—that of gas gangrene; that of malignant œdema; that of botulism; and the organism of tetanus.

The *B. enteritidis sporogenes* is of importance in connection with indications of fæcal contamination of water. In connection with *B. aerogenes capsulatus*, there is some question as to whether the extensive œdema produced by it may not usually be from a terminal or cadaveric infection. At any rate necrotic material seems necessary.

It should be stated that our knowledge of the differential cultural characteristics of anaerobes is unsatisfactory. The exact methods which are in use for aerobes have not been applied to anaerobic organisms.

To Cultivate Anaerobes.—Probably the apparatus giving the most perfect anaerobic conditions is the Novy jar, in which the air has been replaced by hydrogen. The difficulties attending the method are:

FIG. 21.—Novy jar.

1. Unless a special apparatus (Kipp's) is at hand, there may be difficulty in preventing the sulphuric acid from frothing over when poured on the zinc. It should, at first, be added in small quantites at a time—well diluted (1 to 6).

2. Various wash-bottles are required: one containing silver nitrate solution for traces of AsH_3 and one with lead acetate for H_2S and another with pyrogallic acid and caustic soda for any oxygen that may come over.

3. Mixtures of hydrogen and air explode. Consequently, in determining whether all air has been expelled and in its place an atmosphere of hydrogen exists, it is necessary to see if the escaping gas burns with a blue flame. Unless this is collected in a test-tube and examined, we may have an explosion.

4. Except in a large laboratory, where the apparatus is set up and ready for use, too much time would be required.

5. Simpler methods appear to give as good results.

In Tarozzi's method, pieces of fresh sterile organs are added to bouillon. Pieces of kidney, liver, or spleen are best suited. After adding the tissue the media may be heated to 80°C. for a few minutes without interfering with the anaerobic condition producing properties of the fresh tissues. This method is practically the same as that recommended by Smith (see Tetanus). This is also a feature of Noguchi's method of culturing *Treponema pallidum*.

The Method of Liborius

In this it is necessary to have a test-tube containing about 4 inches of a 1% glucose agar. Glucose acts as a reducing agent and furnishes energy. It is convenient to add about 1/10 of 1% of sulphindigotate of soda; the loss of the blue color at the site of the colony enabling us to pick them out. The tube of agar should be boiled just before using to expel remaining oxygen from the tube. Now rapidly bring down the temperature to about 42°C., by placing the tube in cold water, and inoculate the material to be examined. A second or third tube may be inoculated from the first, just as in ordinary diluting methods for plate cultures. Having inoculated the tubes, solidify them as quickly as possible, using tap water or ice-water. The anaerobic growth develops in the depths of the medium. Some pour a little sterile vaseline or paraffin or additional agar on the top of the medium in the tube as a seal from the air. Others have recommended the inoculation of some aerobe, as *B. prodigiosus*, on the surface. This latter method is not advisable. A deep stab culture is often sufficient.

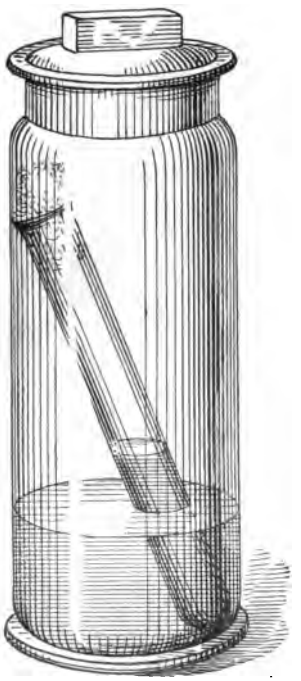


FIG. 22.—Arrangement of tubes for cultivation of anaerobes by Buchner's method. (*Mac Neal.*)

The same technic can be applied to gelatin cultures for anaerobes, pouring in at the completion of the inoculation an inch or so of melted glucose agar to act as a stopper for the gelatin layer below.

The Method of Buchner

In this method 1 gram each of pyrogallic acid and caustic potash or soda for every 100 c.c. of space in the vessel containing the culture is used to absorb the oxygen. It is convenient to drop in the pyrogallic acid; then put in place the inoculated tubes or plates; then quickly pouring in the amount of caustic soda, in a 10% aqueous solution, to immediately close the containing vessel. A large test-tube in which a smaller one containing the inoculated medium is placed, and which may be closed by a rubber stopper, is very convenient. A good rubber-band fruit jar is satisfactory. A desiccator may be used for plates. An excellent method for anaerobic plates, either in a desiccator with the pyrogallic acid and caustic soda, or less satisfactorily in the open air, is to sterilize the parts of the Petri dish inverted; that is, the smaller part is put bottom downward in the inverted cover (as one would set one tumbler in another). Then, in using, unwrap the Petri dish, lift up the inner part, pour in the inoculated medium into the upturned cover. Then immediately

press down the inner dish, spreading out a thin film of the medium between the two bottoms.

Zinsser has originated a very satisfactory method for plate cultures of anaerobes, which is shown in Fig. 7.

Secure two small crystallizing dishes, about 3 and 4 inches in diameter by 1 inch depth and sterilize as for Petri dishes. Pour the inoculated agar into the smaller of the dishes or one can smear the surface of poured glucose agar with the material to be plated out. In the bottom of the larger dish place the dry pyrogallic acid, then invert the smaller dish with the agar surface over it. Quickly pour a 5% solution of caustic soda into the space separating the sides of the inverted smaller dish and the upright larger dish, to a depth of ½ inch and, as it is dissolving the pyrogallic acid, very speedily superimpose paraffin oil on the soda solution to make an air-tight seal.

J. H. Wright's Method

Make a deep stab culture in glucose agar or gelatin, preferably boiling the media before inoculating. Then flame the cotton plug and press it down into the tube so that the top lies about three-fourths of an inch below the mouth of the test-tube. Next fill in about one-fourth of an inch with pyrogallic acid; then add 2 or 3 c.c. of a 10% solution of caustic soda, and quickly insert a rubber stopper. This method is one of the most convenient and practical, and is to be strongly recommended.

Method of Vignal

In this a section of glass tubing (¼ in.) is drawn out at either end, as in making a bacteriological pipette, with a mouth-piece containing a cotton plug. The liquid agar or gelatin is then inoculated and the medium drawn up into the tube by suction with mouth or better with a rubber bulb. In a very small flame the capillary narrowings are sealed off, and we have inside the tube very satisfactory anaerobic conditions. To get at the colonies, file a place on the tube and break at this point.

To obtain material for examination and isolation in pure culture from the deep agar stab-tube, it is best to loosen the medium at the sides of the tube with a heated platinum spud or a flattened copper wire. Then shake the mass out into a sterile Petri dish. It is dangerous to break the tubes with a hammer as some do.

With those anaerobes which produce gas in glucose agar the split in the column of medium enables one to introduce a fine sterile capillary pipette to the site of a colony and by releasing pressure on the rubber bulb to draw up into the tip of the tube material for investigation.

A Combination Method

Recently as shown in the illustration in Fig. 9, I have been combining various methods so that very satisfactory anaerobic conditions are obtained. First, a deep agar stab of freshly sterilized glucose agar is made and inoculated. The surface of this is then covered with sterile paraffin oil. The proper amount of pyrogallic acid is then deposited in a salt mouth bottle. The rubber stopper with the glass and rubber tubing is then firmly pushed in and connection made with a filter pump.

In five to ten minutes almost all the air will be exhausted when the Hofmann clamp is screwed up tight and the bottle disconnected from the vacuum pump. The glass tubing end is then inserted into a graduate holding 10% caustic soda solution, the Hofmann clamp unscrewed, and the necessary amount of caustic soda having been run in, as noted under Buchner method, we again close the screw clamp and incubate.

General Considerations of Pathogenic Anaerobes

Dean and others, working with gas gangrene wounds, have brought out some very practical points in connection with the three important anaerobes found in such wounds, viz.: *B. aerogenes capsulatus, B. œdematis maligni and B. tetani.*

They found an egg broth, made by shaking up the white and yolk of 1 egg in 300 c.c. water a most excellent culture medium. This medium was tubed and sterilized, after which it was liberally inoculated with material from the wounds. After inoculation the tube was heated to 80°C. for one-half hour and then incubated anaerobically. The gas bacillus was present in abundance in such cultures after two or three days' incubation, while the bacillus of malignant œdema later on became the predominant organism. The tetanus bacillus only appeared after a prolonged period —seven to ten days. The bacillus of malignant œdema grew best on Dorsett's egg medium and in two days began to liquefy the slant with a bluish-black discoloration. The growth at first was profuse and creamy white. On glucose agar there was much less gas production than with the gas bacillus. The malignant œdema organisms were as a rule Gram-negative on glucose agar but they were distinctly Gram-positive on Dorsett's medium. The spores were oval in shape, usually located near the end of the rod. The gas bacillus grew well on Dorsett's medium but less vigorously. In glucose agar stabs so much gas was formed that the cotton plug and much of the culture medium tended to be expelled from the tube. The spores form well on Dorsett's medium but not in glucose agar and show as oval bodies distending the central portion of the rod.

Subcutaneous inoculation of the gas bacillus and that of malignant œdema rarely produced death in guinea-pigs.

As regarded the tetanus bacillus they tried various methods of bacteriological diagnosis. The examination of smears from wounds was unsatisfactory in search for "drum-stick" spores. In broth cultures the spores were not present until after several days and in mixed cultures it was difficult to be sure that the terminal spores were those of tetanus and not atypical malignant œdema spores. The best method was to inject guinea-pigs in the subcutaneous tissues of the left chest with 1 or 2 c.c. of mixed broth culture. In two or three days stiffness of the left forelimb was observed soon becoming quite stiff and extended. The spasm extends to other limbs and death occurs in one or two days. There is no evidence of marked inflammation at the site of inoculation.

There have been a number of cases of delayed tetanus often associated with operations done a month or two after the original wound infection so that it is recommended to give antitetanic serum before operation on such cases.

B. Œdematis Maligni (Pasteur, 1877).—This is the vibrion septique of Pasteur. It is found in garden soil and in street sweepings. It is the cause of an acute cellular necrosis attended with serous sanguinolent exudation and with more or less emphysema. The organism only becomes generalized in the blood about the time of death and postmortem. Therefore, it is not a septicæmia, as is anthrax. The bacillus is an organism about the size of anthrax (7μ by 0.8), but is narrower and does not have the same square cut or dimpled ends. Furthermore, it is motile, Gram-negative(?) and an anaerobe. The guinea-pig is very susceptible, and about the time of death and post mortem there may be seen long flexile motile filaments, 15 to 40μ long, which move among the blood-cells as a serpent in the grass (Pasteur).

In cultures it grows out very slightly from the line of stab, giving a jagged granular line, differing from tetanus. Spores form best at 37°C.—requiring about forty-eight hours. It liquefies gelatin. In examining an exudate from a suspected case note the presence of spores centrally situated. Inoculate a guinea-pig. Death occurs in about two days. There is intense hemorrhagic emphysematous œdema at the site of inoculation, the œdematous fluid however does not show spores. The bacilli do not appear in the blood until about the time of death and it is an assistance in diagnosis to put the dead body of the guinea-pig in the incubator for a few hours. The subcutaneous tissue contains fluid and gas. There is present the foul odor of an anaerobe. Examine for the long filaments showing flowing motility. Be sure to stain by Gram. (Negative.) For cultures, heat the material (either from a wound or from a guinea-pig) which shows spores to a temperature of 80°C. for from fifteen minutes to one hour. Then inoculate glucose agar stab culture and grow anaerobically. Courmont differentiates anthrax from malignant œdema by injecting into ear-vein of rabbit. The injection of malignant œdema in this way, instead of subcutaneously, tends to immunize.

B. Botulinus (Van Ermengem, 1896).—This is the organism which produces botulism, a form of meat poisoning. It is a spore-bearing anaerobe and must not be confused with another organism, a non-sporing aerobic bacillus, associated with meat poisoning—the *B. enteritidis* of Gärtner. The spores are at the end and are not very resistant; a temperature of 80°C. often killing them.

In botulism the meat becomes infected after the animal has been slaughtered; in Gärtner meat poisoning the cow meat was infected at the time of slaughter—it

was from a sick animal. Thorough cooking of the meat protects against botulism but not certainly against Gärtner meat poisoning.

There are dysphagia, paralysis of eye-muscles, and cardiac and respiratory symptoms (medulla). The symptoms are due to the elaboration of a soluble toxin

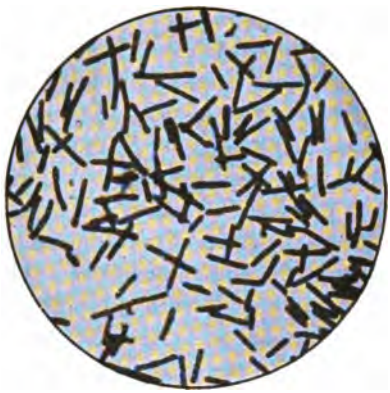


FIG. 23.—Bacillus of botulism. (*Kolle and Wassermann.*)

of the same nature as that of diphtheria and tetanus. There is no fever and consciousness is preserved.

An antitoxin which it is stated has therapeutic value in botulism has been pre-

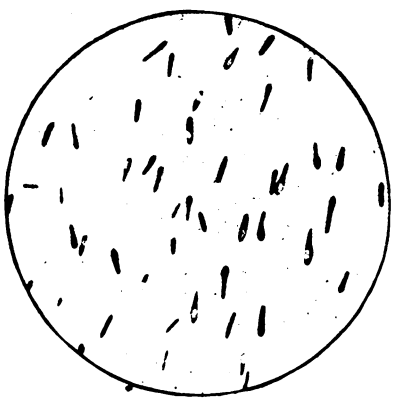


FIG. 24.—Symptomatic anthrax (Rauschbrand) bacilli showing spores. (*Kolle and Wassermann.*)

pared in the usual way by Kempner. Without serum treatment death occurs in about 40% of cases and takes place between twenty-four and forty-eight hours.

The bacillus has been isolated from sausage and ham. It is a rare cause of food poisoning, most of such cases being the result of paratyphoid or enteritidis infections.

It is a large bacillus—5 to 10μ×1μ. It is slightly motile and stains by Gram. It produces gas in glucose media. It grows best at 22° and only slightly at 37°—hence it is dangerous only from its soluble toxin, the bacilli not developing to any extent in the body.

For this reason botulism patients are not a source of danger, it is the infected meat alone which causes the disease. On the contrary where the meat poisoning is due to the Gärtner or paratyphoid group infection may take place from the patient's discharges. Botulism is an intoxication, not an infection. It is not generally known that botulism poisoning may occur from eating infected vegetables as well as meat. In 18 reports of botulism poisoning in the U. S. eleven were traced to canned fruits or vegetables. Of course if such foods were cooked before eating they would be safe but such articles are sometimes eaten raw in salads. Dickson reports the U. S. mortality in 81 cases as 67%.

When the toxin is introduced, it requires a period of incubation of twelve to twenty hours. Symptoms of gastrointestinal disorder may come on shortly after the ingestion of the toxin containing food, these however are not the specific manifestations, as are the eye symptoms, etc.

An important point is that ham may not appear decomposed and yet contain many bacilli and much toxin. It is a very potent toxin—as little as $\frac{1}{1000}$ c.c. may kill a guinea-pig. In man the toxin is apparently absorbed from the alimentary canal, thus differing from most toxins as well as venoms which are usually harmless when introduced by mouth.

For diagnosis inject an infusion of the ham or sausage which was eaten of into a guinea-pig, and characteristic pupillary symptoms with death by cardiac and respiratory failure will result.

Cultures may be made in glucose agar.

The culture is disrupted by gas. Incubation at room temperature and in the dark is necessary. There is a rancid odor. The characteristic point is the production of a powerful soluble toxin which produces symptoms when no bacilli are present.

B. Tetani (Nicolaier, 1885; Kitasato, 1889).—This is the most important organism of the anaerobic spore bearers. Its characteristics are the tetanic symptoms produced by the toxin and the strictly terminal drum-stick spores.

Spores are difficult to find in material from wounds infected with tetanus, but readily develop in cultures. See notes under general consideration of pathogenic anaerobes. Prior to the formation of spores the organism is a long thin bacillus (4×0.4μ). It is motile and Gram-positive. It liquefies gelatin slowly and does not coagulate milk. The stab culture in glucose agar shows pine-tree growth. Colonies on agar plates show as fleecy clouds and microscopically as felted filaments.

Theobald Smith recommends growing it in fermentation tubes containing ordinary bouillon, but to which a piece of the liver or spleen of a rabbit or guinea-pig has been introduced at the junction of the closed arm and the open bulb. By this method spores develop rapidly in from twenty-four to thirty-six hours. Sporulation is most rapid at 37°C. As there is always liability to postmortem invasion of viscera by ordinary saprophytes, Smith recommends that great care be taken not to handle the animal roughly in chloroforming and in pinching off pieces of the organ at autopsy. The animal must be healthy, and the tubes to which the piece of tissue is added must be proven sterile by incubation. Smith calls attention to the uncertainty of the temperature at which tetanus spores are killed. He shows that some require temperature only possible with an autoclave. In view of the danger of tetanus, it is advisable to carefully autoclave all material going into bacterial vaccines, such as salt solution, bottles for holding, etc.

Tetanus seems to grow better in symbiosis with aerobes; hence a lacerated dirty wound with its probable contamination with various cocci, etc., and its difficulty of sterilization, offers a favorable soil. The tetanus bacillus gives rise to one of the most powerful poisons known; it is a soluble toxin like diphtheria toxin, and it is estimated that $\frac{1}{300}$ grain is fatal for man.

There are 2 toxins, tetanospasmin and tetanolysin, but the former is the important one. Tetanus toxin is twenty times as poisonous as dried cobra venom.

The antitoxin is produced by injecting horses with increasing doses of tetanus toxin, following a preliminary dose of 5000 antitoxin units. An important point is that a horse used for the production of diphtheria antitoxin may become infected with tetanus and his blood contain enough tetanus toxin to kill. A number of children in St. Louis died of tetanus as the result of such an accident.

Rosenau has established an antitoxin unit for tetanus which has the power of neutralizing 1000 minimal lethal doses. Practically, it is ten times the least quantity of antitetanic serum necessary to protect the life of a 350 grams guinea-pig from a test dose of tetanus toxin furnished by the hygienic laboratory. (The necessity of some definite unit is apparent when tests have shown that serum stated to contain 6,000,000 units per c.c. only had a value of 90 of the official American units.) Consequently it is a unit ten times as neutralizing as the diphtheria antitoxin one. The antitoxin of tetanus is less efficient than that of diphtheria for the following reasons:

1. There is about three times as great affinity *in vitro* between diphtheria toxin and antitoxin as is the case with tetanus.

2. The tetanus toxin has greater affinity for nerve cells than for antitoxin.

3. Treatment with antitoxin is successful after symptoms of diphtheria appear. With tetanus it is almost hopeless after the disease shows itself. Hence the importance of the early bacteriological examination of material from a suspicious wound (rusty nail).

4. The tetanus toxin ascends by way of the axis cylinder, and the antitoxin being in the circulating fluids cannot reach it, whereas with diphtheria both toxin and antitoxin are in the circulation. Diphtheria also selects the cells of parenchymatous and lymphatic organs which are more tolerant of injury than the nerve cells. The dose of tetanus antitoxin as a prophylactic is 1500 units; as a curative agent 5000 to 20,000 units. Recent experience shows that it should be injected intravenously when symptoms have manifested themselves.

That the disease is due to toxin is shown not only experimentally, but also if spores are carefully freed of all toxin by washing, and then introduced they do not cause tetanus—the polymorphonuclears engulfing them. The importance of the presence of ordinary pus cocci in a tetanus wound may be that the activity of the leukocytes in phagocytizing them allows the tetanus bacillus to escape phagocytosis.

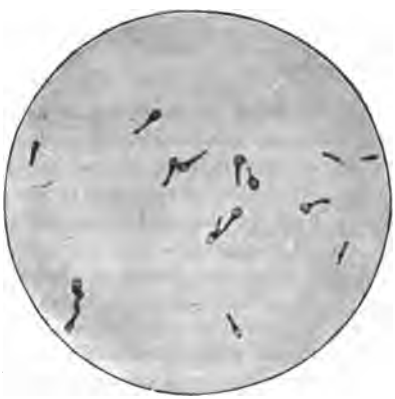


FIG. 25.—Tetanus bacilli showing end spores. (*Kolle and Wassermann.*)

This would also explain the importance of necrotic tissue in a lacerated wound—the phagocytes taking this up instead of tetanus bacilli. The toxin is digested by the alimentary canal juices and infection by that atrium is improbable. The infection occurs especially through skin wounds, and also from those of mucous membrane. While tetanus is like diphtheria, a disease in which the bacilli are localized and do not spread, yet recently Richardson has obtained tetanus bacilli in pure culture from the tributary lymphatic glands of a "rusty nail" wound of foot. The cultures inoculated into root of tail of a white rat caused the rat's death in forty-eight hours with typical "seal gait" attitude of tetanus in rats.

The usual period before symptoms occur is fifteen days. The shorter the period of incubation, the more probably fatal the disease. The horse is the most susceptible animal, next the guinea-pig, then the mouse. Fowls are practically immune.

In examining for tetanus, scrape out the granulation tissue or foreign material from the suspected wound with a sterile Volkmann spoon and insert in a pocket made with scissors in the subcutaneous tissues of the

thigh of a guinea-pig. Animal inoculation is the practical method. One may also put some of the suspected material in a Löffler's serum tube. Place in the incubator, under which conditions the cocci and other aerobes grow luxuriantly and enable the tetanus bacillus to develop. From day to day smell the culture, and if an odor similar to the penetrating, sour, foul smell of the stools of a man who has been on a debauch be detected, it is suspicious. The nondevelopment of a foul odor is against tetanus. Also make smears from the material and examine for drum-stick spores. If these are found, heat the material to 80°C. for one-half hour, to kill nonsporing aerobes and facultative anaerobes, and then inoculate a deep glucose agar tube and cultivate by Wright's method. The fusiform lateral outgrowth about the middle of the stab is characteristic.

A more rapid method is to draw up the material, provided it be pus (tissue scrapings may be emulsified in sterile salt solution) into a capillary bulb pipette. Then seal off the end and heat the capillary bulb pipette and its contents in a water-bath at 80°C. for fifteen minutes. Next break off the sealed tip and stick the pipette into a deep tube of glucose agar. When the point reaches the bottom, force out the material along the line of the stab as the pipette is withdrawn. Cover the surface of the agar with sterile liquid petrolatum and incubate. Better anaerobic conditions obtain where the Buchner or Wright method is employed.

Tetanus produces no gas. Material for examination is best obtained with a bulb pipette (containing a little sterile salt solution) which is plunged into the agar and the salt solution forced out and drawn in where a proper growth is noted.

Spores form in thirty-six to forty-eight hours. In injecting test animals it is advisable to divide the material to be injected into two portions; one animal is injected with the material alone, the second animal with tetanus antitoxin at the same time the material is injected. Only the first animal dies with tetanic symptoms.

Kenneth Taylor states that the search for tetanus bacilli in a wound is usually an unprofitable procedure and even if they rarely can be identified their presence is of little significance.

Serum Treatment.—Taylor notes the inefficiency of serum, however and in whatever doses given, as is also true of carbolic acid and magnesium sulphate when symptoms have set in. The mortality of cases developing within ten days is 80%. As the immunity of a prophylactic injection only lasts about ten days, subsequent operations on an infected area may start up acute tetanus. For this reason a prophylactic injection of about 1500 units should be given such cases prior to operation. Anaphylactic reactions following these secondary operations are rare and these can largely be obviated by subcutaneous injection of desensitizing doses of 0.5 c.c. followed in three hours by the full dose.

Serum sickness showing œdema, urticaria, joint pains, vomiting and malaise may occur in from three to eight days after this injection. It usually lasts two or three days and the prognosis is good.

Chronic Tetanus.—Taylor notes that chronic tetanus is not uncommon. Trismus is frequently absent and the muscles about the wound may show clonic or tonic contractions. A high pulse rate and no fever is rather characteristic. Cases may occur several months after operation or injury especially with low-grade bone infections.

B. Aerogenes Capsulatus (Welch, 1891).—This bacillus is apparently widely distributed. It is possibly the same organism as Klein's *B. enteritidis sporogenes*, which is constantly present in fæces. It is a large capsulated organism, which does not form chains. Spores are produced on blood-serum. These are frequently absent on other media. It is questioned whether its pathogenicity is other than exceedingly feeble, the presence of the bacillus in emphysematous findings at postmortem being attributed to terminal or cadaveric invasion.

Cases, however, in the Philippines, have been reported following carabao horn wounds, in which most serious and fatal results attended emphysematous lesions showing this bacillus. The isolation of a Gram-positive bacillus from a lacerated wound discharge, even in the absence of emphysema, is almost diagnostic.

In milk cultures we have coagulation and from the subsequent development of gas the disruption of the coagulum into shreds. An odor of butyric acid is developed.

Cultures in litmus milk show these shreds plastered against the sides of the tube and showing a pink color.

It is the cause of "foamy organs" occasionally present at autopsy.

The best method of diagnosis is to inoculate the culture or material into the ear vein of a rabbit, kill it and then incubate the body at 37°C. Gas is generated in the organs in a few hours. While the organism is pathogenic for guinea-pigs it has little effect on rabbits.

At the American Ambulance a sterile cotton throat applicator is used to obtain the discharge from a wound. Each swab is sent to the laboratory in a tube. A glucose agar tube is boiled for ten minutes, then quickly cooled to 42°C. The swab is then introduced into the melted agar and well rubbed up in it. This deep agar stab is then quickly solidified in ice water and put in the incubator. For quick diagnosis the swab, coated with agar, is transferred to the tube from which it was taken and this latter is placed in a larger tube containing pyrogallic acid and sodium hydroxide (anaerobic culturing). In four or five hours a smear can be made from this swab and stained by Gram to note presence of the gas bacillus.

B. perfringens is the name frequently given to the organism which has assumed such great importance on account of its causing the gas gangrene so frequently observed in shell wounds received in Belgium. It is considered to be identical with *B. aerogenes capsulatus* (Welch's gas bacillus) and *B. phlegmonis empysematosæ.* It is very abundant in the soil of highly fertilized areas. Statistics have shown that from 70 to 90% of wounds on the Flanders front are infected with the gas bacillus.

FIG. 26.—*B. aerogenes capsulatus* agar culture showing gas formation. (*Mac Neal.*)

In size the bacillus is large, 6×2.5 microns with square cut ends. It is strongly Gram-positive, may or may not show a capsule and is nonmotile. When sporing the spore occurs toward one end with slight bulging of the rod. Grown in pure culture spores are rarely found, but in symbiosis with staphylococci they form abundantly. Cultures from the clothing of men in the trenches almost always show the Welch bacillus and less frequently the tetanus bacillus. Streptococci were rather frequent. When a shell wound occurs we almost invariably have a gas bacillus infection which during the first few days gives rise to a foul-smelling reddish-brown discharge. Smears from gas gangrene wounds, showing such discharge, have chiefly the gas bacillus and streptococci. In the second week the pus becomes more purulent and the gas bacillus is infrequent. Streptococci, staphylococci and coliform bacilli are abundant. Glucose agar to which about $\frac{1}{10}$ c.c. of blood has been added makes a very favorable medium for the gas bacillus. It also grows well on milk or blood-serum. Fleming prefers neutral red egg medium for its culturing. Cultures of the gas bacillus from gas gangrene discharges when injected subcutaneously into guinea-pigs kill within twenty-four hours causing emphysematous swellings. Chlorinated solutions seem to be more efficient against the infection than the formerly recommended hydrogen dioxide.

In studying the pathogenic effect of the gas bacillus, Taylor considered the gas as of little toxic importance. The mechanical effect

of pressure produced is the most important part of the infection causing death of tissues from pressure on blood-vessels. It also causes fragmentation of muscle tissue and scattering of the infection. In treatment provision must be made for escape of gas.

Bull and Pritchett insist on the importance of a soluble toxin (exotoxin) which produces both hæmolysis and necrosis. By injecting rabbits with this soluble toxin they have produced an antitoxin. McGlennan has used this antitoxin (about 7500 units intravenously) in three cases of gas gangrene infection with two recoveries. It is a fact that Dakin's solution will destroy the toxin of the Welch bacillus.

Achalme isolated a large bacillus from a fatal case of rheumatism which is now considered as having no relation to acute rheumatism and which was probably *B. aerogenes capsulatus*.

Kendall has called attention to the importance of this organism in a certain proportion of cases of summer diarrhœa of infants. (See under chapter on Fæces.)

CHAPTER VII

STUDY AND IDENTIFICATION OF BACTERIA. MYCOBACTERIA AND CORYNEBACTERIA. KEY AND NOTES

Key for Bacilli.—Having branching characteristics, as shown by parallelism, branching, curving forms, V-shapes, clubbing at ends, segmental staining, etc.

Acid-fast. Mycobacterium. { Cultures more or less wrinkled and dry. / More like moulds.

I. Grow rapidly on ordinary media at room temperature.
Examples: Timothy grass bacillus of Moeller (B. phlei).
Mist bacillus. Butter bacilli as reported by (1) Rabinowitsch and (2) Petri.

II. Only grow at about incubator temperature. Scanty growth or none at all on ordinary media. Media of preference are: (a) solidified blood-serum, (b) glycerine agar, (c) glycerine potato and (d) egg media.

1. Cultures fairly moist, luxuriant, and flat. Op. temp. 43°C.
a. Bacillus of avian tuberculosis.

2. Cultures scanty, wrinkled, and dry. Appear in ten to fourteen days. Op. temp. 38°C. Bacilli longer, narrower, more regular in outline and staining than bovine; vacuolation more marked (2.5μ). Smear from organs of inoculated guinea-pig shows few bacilli. Less virulent for rabbits.
a. Bacillus of human tuberculosis.
Cultures as above, but even more scanty. Bacilli shorter, thicker, less vacuolated (1.5μ). Smear from organs of guinea-pig shows many bacilli.
b. Bovine tubercle bacilli.

3. Very difficult to cultivate (Czaplewski).
Smegma bacilli for various animals.

III. Noncultivable by ordinary methods. Cultivable in symbiosis with amœbæ. (Clegg.) Duval cultivated an acid-fast bacillus on N.N.N. medium containing 1% glycerine. Bayon cultivated on placental juice glycerine agar a slightly acid-fast diphtheroid which changed to acid-fast in peritoneum of mouse. Bayon's organism thought to be similar to Kedrowsky's diphtheroid of leprosy.

1. B. lepræ. Found chiefly in nasal mucus and in juice from lepra tubercles. Less often in nerve leprosy.

Nonacid-fast. Corynebacterium. { Colonies more flat and moist. Like other bacteria.

I. Do not stain by Gram's method.

1. B. mallei (Glanders). Characteristic culture is that on potato. Growth like layer of honey by third day. Becomes darker in color, until on eighth day is reddish brown or opaque with greenish-yellow margin.

II. Gram-positive.

1. Very luxuriant growth on ordinary media. Colonies often yellow to brownish. B. pseudodiphtheriæ. Shorter, thicker and stain uniformly.
2. Moderate growth on ordinary media. B. diphtheriæ. Best media are blood-serum (Löffler's) or glycerine agar. Has metachromatic granules at poles.
3. Scanty and slow growth on nutrient media. B. xerosis.

The Group of Acid-fast Branching Bacilli

There is a large and ever-increasing number of organisms which have the same staining reactions as the tubercle bacilli, but which differ in four important essentials of:

1. Growing readily on any media.

2. Showing more or less abundant growth or colonies in twenty-four hours.

3. Having no pathogenic power for guinea-pigs when inoculated subcutaneously.

4. Not requiring body temperature for development, but growing at room temperature.

Tuberculosis in the Guinea-pig.—Many of these organisms if injected intraperitoneally into guinea-pigs will produce a peritonitis with false membrane. Some also produce granulation tissue nodules which may be confused with true tubercles. For this reason it is well to study the lesions in experimental tuberculosis in the guinea-pig. Injected subcutaneously, on either or both sides of the posterior abdomen with the needle pointing toward the inguinal glands, we may have caseation and ulceration at the site of inoculation. The glands in relation enlarge and caseate. Smears from these show T. B. The marked and characteristic change is the enormous enlargement of the spleen, which is studded with grayish and yellow tubercles. Make smears and cultures from the spleen. The death of the guinea-pig usually occurs in about two months. The lesions may be looked for at three to five weeks.

The nonpathogenic acid-fast bacilli are of greatest importance by reason of their possible confusion with the true tubercle bacilli. Their colonies correspond more or less with different types of tubercle bacilli colonies, being either dry and wrinkled like human, or moist and irregularly flat as avian. Eventually the moist colonies become dry and wrinkled. They have been isolated from:

1. Butter and milk.
2. From grasses, especially in timothy grass infusion.
3. In various excretions of animals, as in dung, urine, etc.
4. Normally in man—from skin, nasal mucus, cerumen, and tonsillar exudate.

FIG. 27.—*Bacillus tuberculosis;* glycerine agar-agar culture, several months old. (*Curtis.*)

It is important to remember that such organisms have very rarely been reported from pulmonary lesions, and when present they have been considered as probably causative.

The present view is that the finding of tubercle bacilli in sputum has practically as great value as it had before we knew of these various acid-fast bacteria.

Tubercle Bacillus (Koch, 1882).—This is a rather long, narrow rod, $3 \times 0.3\mu$. In the human type it tends to show a beaded appearance, this not being due to spores, however. In the bovine type the staining is more solid, the organism shorter and thicker, and shows even a more scanty growth than human T. B. It has been established that many of the tuberculous affections of man, especially those of the skin, bone, and mesenteric glands, are of the bovine type, while, as a rule, pulmonary and laryngeal lesions are of the human type. Experiments by various commissions in different countries have shown that human and bovine types are very closely related and that not only may a bovine strain affect man, but that human T. B. may infect young calves. As bacilli of the bovine type have frequently been reported in intestinal and mesenteric tuberculosis of children it shows the importance of sterilizing cows' milk. Koch considered human infection from bovine sources as of very rare occurrence.

Although Kossel has found only two cases of bovine T. B. in 709 cases of pulmonary tuberculosis yet for the other types the findings are different. Leaving out of consideration the frequency of infections with bovine T. B. in children, recent statistics have shown that in adults about 4% of cervical adenitis, 22% of tabes mesenterica and 3.5% of bone and joint tuberculosis are due to bovine strains of T. B.

Park and Krumweide, in a study of more than 1000 cases, found about 10% due to bovine tuberculosis. Of 686 adult cases only 1.3% showed bovine strains while 352 cases under sixteen showed approximately 25% of bovine infection. Of 592 cases of pulmonary tuberculosis, in children and adults, not a single case could surely be regarded as bovine.

Atrium of Infection.—A subject of great moment is that of the atrium of infection in tuberculosis. While 75% or more of human cases are of the respiratory tract, yet we now have views that Cornet's idea of T. B. containing dust being aspirated, or Flügge's spray method of infection from droplets of sputum in coughing, may be but rarely operative. The path from intestinal tract to thoracic duct and lungs is direct, so that tuberculous bronchial glands of lung infection may be by way of intestines. Some European statistics, using von Pirquet's method, have shown 90% of children under fourteen infected while similar American ones have shown about 50%. The prevailing idea is that we get our infection in childhood and show the disease as we approach adult life. Infection after adult life is exceedingly rare, as shown by the rarity of the disease in attendants at institutions for the tuberculous. The respiratory atria would be just as operative for adults as children so the probable explanation is that children in crawling about, where tuberculous material is accessible, may contaminate the fingers and have infection take place by the digestive tract. With bovine infections we are sure that infection of man takes place almost exclusively by the alimentary tract.

Types.—The British Royal Commission in its final report of July, 1911, considered three types of T. B.

I. The bovine type belonging to the natural tuberculosis of cattle.

II. The human type. The type more generally found in man.

III. The avian type, belonging to natural tuberculosis of fowls.

The bovine type grows slowly on serum and at the end of two or three weeks shows only a thin grayish uniform growth which is not wrinkled and not pigmented. The human type grows more rapidly and tends to become wrinkled and pigmented. Subcutaneous inoculation of 50 mg. of culture into the neck of calves produced generalized tuberculosis. A similar injection of human T. B. does not cause generalized tuberculosis but only an encapsulated local lesion.

Intravenous injection of 0.01 to 0.1 mg. of bovine T. B. into rabbits causes general miliary tuberculosis and death within five weeks. With human T. B. in doses of 0.1 to 1.0 mg., similarly injected, the majority of rabbits live for three months.

Subcutaneous injection of 10 mg. bovine T. B. causes death in 28 to 101 days. Similar injection of human T. B. in doses up to 100 mg. did not kill the rabbits after periods of from 94 to 725 days. The duration of life in injected guinea-pigs is longer with human than with bovine T. B.

Subcutaneous injections of bovine T. B. into cats produces generalized tuberculosis while the cat is resistant to human T. B. thus given.

Recent statistics (Beitzke) show tuberculous lesions in 58% of adults at autopsy—Naegli's figures were about 90%.

Avian Type.—It is a question whether the avian type is absolutely distinct; many experiments having indicated the impossibility of infecting fowls with human T. B. Nocard, by inserting collodion sacs containing bouillon suspensions of human T. B., claims to have changed these to the avian type. The avian type grows at 43°C. fairly luxuriantly, as a moist, more or less spreading culture. It grows much better on glycerinated agar than on serum. Morphologically they are like the human type, but show less tendency to form compact masses. Very pleomorphic. Have been reported from sputum of man (doubtful).

Fowls become infected by intravenous or subcutaneous injection or as the result of feeding. After feeding the lesions are chiefly of the alimentary tract; after injections, of spleen, liver and lungs. Avian T. B. is more virulent for rabbits than human T. B. but less so than bovine T. B. The mouse is the only animal besides the rabbit in which avian T. B. can cause a generalized tuberculosis. The conclusions are that there is no danger to man from avian T. B. With the bovine type it is quite different as nearly one-half of the deaths in young children from abdominal tuberculosis were due to bovine T. B. and to that type alone. Not only in children, but in adolescents suffering from cervical gland tuberculosis, a large proportion were caused by bovine types. The bovine type is also an important factor in lupus.

There is also a *fish tuberculosis*. This organism grows much more rapidly than the other types (three to four days), and grows best at 24°C., growth ceasing at 36°C. The colonies are round and moist.

It is certain that many of the symptoms usually noted in the tuberculous are due to secondary infections. Pettit, by careful blood cultures, obtained the *Pneumococcus* in 24 cases and the *Streptococcus* in 36 cases out of 130 cases studied. He used from 5 to 20 c.c. of blood from the vein. Positive blood cultures were obtained in 68% of far-advanced cases, 45% of advanced cases and 16% of incipient cases.

Culture Media.—The best culture medium for primary cultures is blood-serum or, better, a mixture of yolk of egg and glycerine agar. Petroff's medium and Dorsett's egg medium are also used. In subcultures, either glycerine agar, glycerine potato, or glycerine bouillon make good media.

In inoculating media from tuberculous material, as, say, from a tuberculous gland or, more practically, from the spleen of a guinea-pig, the material must be thoroughly disintegrated or rubbed on the surface of the media so that individual bacilli may rest on the surface of the culture media. In growing in flasks in glycerine bouillon a surface growth is desired. The cylindrical flask of Koch gives a better support to the pellicle than an Erlenmeyer one. In inoculating, a scale of such a surface

growth on a grain from the growth on a slant should be deposited on the surface of the glycerine bouillon in the flask. In cultivating from sputum use Petroff's medium.

Toxic Products.—Inasmuch as the filtrate from cultures has little toxic effect, the poison is assumed to be intracellular.

Tuberculins.—Koch's "Old Tuberculin," which was simply a concentrated 5% glycerine bouillon culture, is now principally used in diagnosis. It was prepared as follows:

After four to six weeks the surface growth begins to sink to the bottom of the flask. This fully developed culture is evaporated over a water-bath at 80°C. to one-tenth the original volume. It is then filtered, the final product containing about 40% of glycerine.

Koch's "New Tuberculin" or tuberculin "R" was introduced in 1897. In this, virulent bacilli are dried *in vacuo* and ground up until stained smears fail to show intact bacilli. One gram of such material is triturated with 100 c.c. water and centrifugalized. The supernatant fluid is removed and is designated "T. O." The residue is then dried, triturated in water and centrifugalized. Subsequent trituration and centrifugalization, preserving each time the supernatant suspension, gives the new tuberculin. It has been found at times to contain virulent T. B.

Koch's bazillen emulsion has been more recently introduced by Koch (1901). This is simply a suspension of ground-up bacilli in 50% glycerine solution. It really is "T. O." and "T. R." combined and contains 5 mg. of bacillary substance in 1 c.c. Another preparation is the bouillon filtrate of Denys. This is the unheated Chamberland filtrate of broth cultures of human T. B. It contains ¼% phenol.

In the use of T. R. and of bazillen emulsion, Sir A. Wright recommends doses of $\frac{1}{4000}$ mg., and he rarely goes beyond $\frac{1}{1000}$ mg. in treatment. These products come in 1 c.c. bottles containing 5 mg. of bacillary material. It is convenient to remove $\frac{3}{10}$ c.c., containing 1 mg. Add this to 10 c.c. of glycerine salt solution with ¼% of lysol. Each c.c. contains $\frac{1}{10}$ mg. One c.c. of this stock solution added to 99 c.c. of salt solution, with ¼% of lysol, would give a working solution, each c.c. of which would contain $\frac{1}{1000}$ mg. of tuberculin.

Diagnosis.—For diagnostic tuberculin reactions we have the following:

1. Subcutaneous injection of $\frac{1}{5}$ mg. If no reaction occurs in four or five days we may increase to 1 to 5 mg.

Positive reactions show (*a*) constitutional symptoms of fever, malaise and possibly chill; (*b*) focal symptoms, as when a tuberculous gland, joint or skin involvement becomes active, and (*c*) local reaction as shown by the tenderness, induration or inflammation at the site of injection.

2. Variations in opsonic index.

3. Instillation into one eye of a drop of ½% or 1% solution of purified tuberculin.

Reaction is shown by redness, especially of inner canthus, in twelve to twenty-four hours (Calmette). A previous instillation may sensitize a nontuberculous case and a second application of the drop may give an erroneous diagnosis. This test should not be used in persons over fifty or when there is any disease of the eye to be used or for that matter of the other eye. For instance, in corneal opacities, due to T. B. keratitis, a focal reaction would occur.

4. The cutaneous inoculation method (similar to ordinary vaccination methods). Scarify two small areas on the arm (1/10 inch in diameter) about 2 inches apart. Rub in one a drop of old tuberculin, in the other a drop of 25% tuberculin. As a control scarify a spot midway and to one side of the others and rub in 1 drop of 0.5% carbolic glycerine. The appearance of bright red papules in twenty-four hours indicates reaction (von Pirquet).

It is now recommended to deposit a loopful of undiluted tuberculin on the skin and below that a loopful of saline. A linear incision with a sharp scalpel or glass needle is made through the saline and then through the drop of tuberculin trying not to draw blood. After two to five minutes the tuberculin is wiped off. No dressing is required. Reaction usually appears as induration or inflammatory areola or vesicles after twenty-four hours, but may be delayed forty-eight hours. This is the method of preference.

5. Intracutaneous inoculation of 1 drop of a 1–1000, 1–100 or 1–10 dilution of old tuberculin (Mantoux and Moussu).

Webb recommends hypodermic needle points which have been dipped in old tuberculin and the points allowed to dry. A drop of water is placed on the skin and the needle points having been moistened in it are plunged through the skin and withdrawn with a twist. A definite lump shows a positive reaction.

6. Ointment tuberculin test. Rub in 50% ointment of tuberculin in lanolin. Reaction is shown by dermatitis with reddened papules in twenty-four to forty-eight hours (Moro).

7. Inoculation of bovine and human tuberculin to diagnose type of infection (Detre). Of questionable value.

Laboratory Diagnosis.—Although we can usually make a diagnosis by staining sputum or other tuberculous material it is more positive to inject a guinea-pig with the material. See under section on group of acid-fast bacilli.

It will be noted that a small indurated nodule appears in about ten days at the site of inoculation of the guinea-pig. This softens and forms an abscess communicating with an ulcerated area, the tuberculous chancre. By squeezing this ulcerated area we may get a discharge which, when stained, shows tubercle bacilli.

Clough has recently noted the value of blood cultures on Petroff's medium in cases of miliary tuberculosis. Such findings are practically negative in other forms of tuberculosis (6.7%), but in miliary tuberculosis the findings were 66.6%.

Ebright injects the suspected material into the subcutaneous tissue of one side of the abdomen of 3 guinea-pigs. At the end of one week an injection into the other side of the abdomen of one of the guinea-pigs of ¼ c.c. tuberculin is given. Twenty-four hours later smears are made from the original site of inoculation and examined for tubercle bacilli. If negative this is repeated with a second guinea-pig at the end of the second week and finally at the end of the third week with the third guinea-pig.

Bloch's method is to damage the lymphatic glands in the inguinal region by squeezing the tissue between the fingers. Injections made there of tuberculosis material show abundant tubercle bacilli in these damaged glands in ten to twelve days.

In staining it is better to use the Ziehl-Neelsen method, decolorizing with 3% hydrochloric acid in 95% alcohol. The alcohol, for all practical purposes, enables us to eliminate the smegma and similar bacilli, these being decolorized by such treatment. There are two objections to the Gabbett method, where decolorizer and counterstain are combined: 1. We cannot judge of the degree of decolorization—we are working in the dark; and 2. the matter of elimination of smegma bacilli is impossible.

Pappenheim's method, in which corallin and methylene blue are dissolved in alcohol, does not appear to have an advantage over acid alcohol. As a practical point when the question of tuberculosis of the genito-urinary tract is involved, inoculate a guinea-pig with urinary sediment.

It must be remembered that in young cultures of tubercle bacilli many of the rods are nonacid-fast, taking the blue of the counter stain, while older rods are acid-fast. This frequently causes suspicion of a contaminated culture.

Discussion has arisen as to the granules of Much. These are considered by Much as resistant forms while others consider them degeneration forms of tubercle bacilli. At any rate material containing only these Gram-positive granules and no acid-fast rods may when injected into animals give rise to tuberculosis and acid-fast bacilli.

The combination of the acid-fast and Gram-staining methods as recommended by Fontes is very satisfactory.

Bacillus Lepræ (Hansen, 1874).—This is the cause of leprosy. In nodular leprosy the organism is readily and in the greatest abundance found in the juice of the tubercles of the skin, and secretions of ulcerations of nasal and pharyngeal mucosa.

The earliest lesion is probably a nasal ulcer at the junction of the bony and cartilaginous septum. Scrapings from this ulcer may give an early diagnosis.

In the skin they are chiefly found in the derma packed in the so-called lepra cells. The process is granulomatous but does not show the caseation of tuberculosis or the predominant plasma cells of syphilis. The bacilli are also found engulfed in the endothelial cells lining the lymphatics.

They are also found in the glands in relation to the superficial lesions. The bacilli are found in smaller numbers in the liver and spleen. In anæsthetic or nerve leprosy they are found in small numbers in the granuloma tissue which affects the interstitial connective tissue of the peripheral nerves. Also, rarely, in the anæsthetic spots of nerve leprosy.

The leprosy bacilli are found in profusion in the granulomatous tissue of the corium and subcutaneous structures of the leprous nodules, chiefly within cells called "lepra cells" and also within endothelial and connective-tissue cells as well as lying free, packed in lymphatic channels, the so-called "globi."

The leprosy bacillus may be distinguished from the tubercle bacillus by the following points:

1. The presence ordinarily of huge numbers of bacilli often grouped in packets like a bundle of cigars tied together. It will be remembered that it is very difficult to find even a single tubercle bacillus in a skin lesion. Leprosy bacilli form palisade groups but not chains.

2. The leprosy bacilli stain more solidly and when granules are present they are coarser and more widely separated than the fine granulations of the tubercle bacillus.

3. They do not stand decolorization quite as well as the tubercle bacillus. With 20% sulphuric acid in water they hold their color almost as well as tubercle bacilli but with 3% HCl in alcohol they decolorize in about two hours as against twelve to twenty-four hours for the tubercle bacillus.

4. Leprosy bacilli have neither been surely cultivated nor surely inoculated with pathogenic results into guinea-pigs or other experimental animals and it is by the negative results upon cultivating or animal inoculation that we have our surest method of differentiation from tubercle bacilli.

Leprosy bacilli are chiefly spread through the lymphatics, but in nodular leprosy, their occurrence in the blood stream during the febrile accessions is so constant that

this route may also be of importance. Next to the corium they are most abundant in the lymphatic glands. They stain readily by Gram's method.

Cultivation Experiments.—A great amount of work has been done within recent years in attempting to cultivate the leprosy bacillus.

In 1900 Kedrowsky, culturing material from 3 cases of leprosy obtained diphtheroids from 2 and a streptothrix from 1. A rabbit was inoculated first intravenously and later intraperitoneally with this nonacid-fast streptothrix and when killed six months later showed peritoneal nodules from which both diphtheroids and acid-fast bacilli, but not a streptothrix, were recovered culturally. Injection of cultures of the acid-fast bacilli and diphtheroids into rabbits and mice produced nodules which, when cultured, showed acid-fast organisms or diphtheroids. In 1901, he cultivated a diphtheroid from a fourth case of leprosy.

Fraser and Fletcher working with Kedrowsky's culture produced peritoneal nodules with the killed as well as the living organism. They were able to produce the same results with *B. phlei.*

With emulsions of leprous nodules, rich in leprosy bacilli, they could not produce similar lesions in the experimental guinea-pigs.

Rost obtained a culture on a salt-free medium from which he prepared his leprolin by a process similar to that used for old tuberculin. It was claimed that leprolin had marked curvative power in leprosy. Recently Williams and Rost have cultivated a streptothrix on a medium containing milk.

Clegg, by inoculating his medium with cultural amœbæ, obtained growth of a diphtheroid organism, with acid-fast tendencies, from the spleen pulp of lepers.

Duval, by using media containing amino-acids, as result of tryptic digestion, brought forward two organisms, one of which was a diphtheroid and grew luxuriantly while the other showed a slow scanty growth and was acid-fast.

Bayon, by using placental media, isolated an organism rather resembling that of Kedrowsky. These organisms alone responded to immunity tests when such were made by Bayon and they alone gave rise to tissue changes resembling those of leprosy when injected into animals.

Professor [illegible]rie obtained a streptothrix-like growth from the granulomatous tissue of excised [illegible]us nodules. The ethereal extract from this culture gave a neutral fat which he called nastin and which is the basis of a leprosy treatment.

Quite recently and after working for eighteen months, with material from 32 nonulcerative cases of nodular leprosy, not only with media as recommended by Duval, Rost and Bayon, but with blood and serum culture media, both by aerobic and anaerobic procedures, Fraser has been unable, in a single instance, to obtain any evidence of growth from this wealth of leprosy material.

As being opposed to the possibility of culturing the human leprosy bacillus, it may be stated that most of the experiments along this line with rat leprosy, a disease occurring naturally in rats and caused by an organism almost identical, as to lesions produced, with the leprosy bacillus, have been negative. Bayon, however, states that he has cultivated the bacillus of rat leprosy.

Recently a *leprosy-like disease of rats* has been reported in which there are two types: 1. A skin affection and 2. a glandular one. In this disease, acid-fast bacilli, alike in all respects to leprosy bacilli, have been found. Deane has obtained a diphtheroid-like organism in culture, which is nonacid-fast. This same finding has been obtained in cultures considered positive in human leprosy.

There have been many reports of positive findings with the Wassermann test in cases of tubercular leprosy but such reports are considered doubtful by many. Butler, in the Philippines, has found that the lepers gave no higher percentage of positive Wassermann reactions than did the nonleprous native patients at his clinic.

Transmission.—There is nothing definitely known as to method of transmission of the disease.

In rat leprosy it has been found that infection of other rats takes place as readily through slight abrasions of the skin as when material is injected subcutaneously.

The idea is that natural infection occurs by way of the skin and through the lymphatics. There is no evidence that insects play a part in transmission.

Rat leprosy prevails extensively in Europe, Asia and America. Although similar etiologically and pathologically there does not seem to be any connection between the disease in rat and in man; as is the case with human and rat plague.

Laboratory Diagnosis.—The usual procedure is to scrape a spot or nodule with a scalpel until the epidermis has been gone through and then smear out the serous exudate on a slide and stain by the Ziehl-Neelsen acid-fast method or by Gram's stain. Twenty percent sulphuric acid is less apt to decolorize than the 3% acid alcohol, the leprosy bacilli being less resistant to acid alcohol decolorization than to aqueous acid solutions. There is a great variation in the resistance to decolorization of leprosy bacilli, a preparation from one case holding its color almost as well as tubercle bacilli, while material from another case may decolorize very easily.

I am partial to Tschernogabow's technic. In this one punctures the subepithelial granulomatous tissue with a capillary pipette, the end of which has been broken off by tapping the point in order to give a cutting point, and the serum which exudes is smeared out and stained.

Some prefer emulsifying a piece of the tissue and centrifuging and staining the sediment. Quite recently the antiformin method of treating leprous tissue, as for tuberculous tissue, has been used.

Many insist that the best method is to cut out small sections of the lesion, going well into normal tissue, and putting through paraffin and cutting thin sections and staining. Gram's method, counterstaining with Bismarck brown gives beautiful preparations. For acid-fast staining first stain with hæmatoxylin to obtain a histological background and then steam with carbol-fuchsin, decolorize very briefly with acid alcohol, then through absolute alcohol and xylol.

Of the greatest diagnostic value is the staining of the nasal mucus or scrapings from ulcerations on nasal septum for leprosy bacilli. These are often found in the characteristic cigar package bundles or engulfed in lepra cells. A standard procedure is to give 60 grains of iodide of potash to cause a drug coryza, in the secretions of which leprosy bacilli may be found. However, one will have better success if the nasal secretion be obtained at a time when a natural coryza exists.

Thibault examined the nasal mucus, gland juice and blood of 30 lepers. He obtained leprosy bacilli in the nasal mucus of 20, in the gland puncture juice of 18, and in the blood of 7.

Hollman detected leprosy bacilli in the nasal mucus of 90% of 58 nodular cases, of 67% of 6 mixed leprosy and of 45% of anæsthetic cases, after making 329 examinations.

Leprosy bacilli are apt to be found in the blood of nodular cases, especially at the time of the febrile accessions. The blood is best taken in 5 or 10 c.c. quantities into 1% sodium citrate in distilled water. After centrifuging, the sediment is treated with 10% antiformin, at 37°C. for one hour. Again centrifuging, and washing, the sediment is smeared out on a slide and stained. The bacilli are not apt to be found in the blood of cases of nerve leprosy.

Gland puncture has recently been considered as an important diagnostic procedure in leprosy.

It must not be forgotten that while the finding of leprosy bacilli is usually very easy in the nodules of nodular leprosy it is a painstaking and discouraging procedure with the spots of nerve leprosy. Even the affected nerves, at autopsy, often fail to show bacilli. For nerve-leprosy the examination of nasal mucus is of prime importance.

The X-ray has been utilized in the recognition of the very early, trophic changes in bone, showing the commencing absorption of phalanges.

Nonacid-fast Branching Bacilli

Bacillus Mallei (Löffler and Shutz, 1882).—This is the cause of a rather common disease of horses. When affecting the superficial lymphatic glands, it is termed "farcy;" when producing ulceration of nasal mucous membrane, the term "glanders" is used.

In man there are 2 types of glanders—chronic and acute. In the chronic form an abrasion becomes infected from contact with glanders material and an intractable foul discharging ulceration results. This may persist for months with lymphatic involvement or may become acute. The acute form may also develop from the start and the cases are usually diagnosed as pyæmia. There is great prostration

with marked pains of the extremities. Death invariably results in acute glanders. The bacillus is a narrow, slightly curved rod, about $3 \times 0.3\mu$. It is nonmotile and Gram-negative. It at times presents a beaded appearance. In subculture on agar or blood-serum the growth is somewhat like typhoid but more translucent. In original cultures from pus or tissues the colonies may not show themselves for forty-eight hours.

As the organism does not tend to invade the bood stream, blood cultures are apt to be negative. The glanders bacillus grows best on an acid glycerine agar (+2).

The characteristic culture is that on potato. Grown at 37°C., we have a light brown or yellowish honey-like or mucilaginous growth, which by the end of a week spreads out and takes a cuprous oxide like reddish tint with greenish borders. The potato assumes a dirty brown color. This and the inoculation of a guinea-pig are the chief diagnostic measures. If the material is injected intraperitoneally into a male guinea-pig, marked swelling of the testicles is noted within forty-eight hours, at the earliest, to seven to ten days. Cultures should be made from this swollen testicle as other organisms than glanders may bring it about.

Only the *B. pyocyaneus* and cholera vibrios give a similar coloration of potato. These organisms, however, are easily differentiated. The glanders bacillus is the most dangerous of laboratory cultures and should be handled with extreme care.

The best stains are carbol thionin and formol fuchsin. In sections stained with carbol thionin the bacilli are apt to be decolorized by the subsequent passage of the section through alcohol and xylol. This may be avoided by blotting carefully after the thionin, then clearing with xylol or some oil and mounting. Nicolle's tannin method is a good one.

Mallein is prepared by sterilizing cultures that have grown in glycerine bouillon for about a month by means of heat (100°C.). The dead culture is then filtered through a Berkefeld filter and the filtrate constitutes mallein. It is chiefly used as a means of diagnosing the disease in horses. The reaction consists in rise of temperature and local œdema. The dose is about 1 c.c.

Agglutination and complement-fixation tests are also used for diagnosing glanders.

Bacillus Diphtheriæ (Klebs discovered, 1883; Löffler cultivated, 1884).—The diphtheria bacillus is found not only in the false membrane which is so characteristic of the disease, but may be found in abundance in the more or less abundant secretions of nose and pharynx. In studying the epidemiology of diphtheria, especial attention must be given to the examination of nasal discharges.

Carriers.—With diphtheria carriers it is important to remember that the crypts of the tonsils may harbor the bacilli and thus protect them from the ordinary application of antiseptic agents. Goldberger found that the combination of throat and nose cultures gave much higher findings with carriers than either separately. The

nose cultures gave more positives than the throat ones. These workers only obtained about 1% of positives in 4093 cases in Detroit, these being lower than figures

FIG. 28.—Bacillus of diphtheria. (×1000.) (*Mac Neal.*)

from other sources. It is interesting to note that 32% of these people showed pseudo-diphtheria bacilli.

FIG. 29.—Diphtheria bacilli involution forms. (*Kolle and Wassermann.*)

Infection of the larynx and middle ear are not very rare. The mucous membrane of the vagina or the conjunctiva may also be infected. The *B. diphtheriæ* may be in pure culture lying entangled in the fibrin

meshes or contained within leukocytes in the membrane or be associated with staphylococci, pneumococci, or especially streptococci. These latter complicate unfavorably and cause the suppurative conditions about the neck. In fatal cases the diphtheria bacillus may be found in the lungs. Ordinarily, however, it remains entirely local and does not get into the circulation or viscera.

Toxic Products.—It produces soluble absorbable poisons which are designated toxin in the case of the one responsible for the acute intoxication, parenchymatous degeneration and death and toxone for the poison which produces œdema at the site of inoculation and postdiphtheritic palsy. The injection of the soluble poisons alone without the bacilli produces the symptoms of the disease.

FIG. 30.—*B. diphtheriæ* stained by Neisser's method. (*Mac Neal.*)

Morphology.—The bacilli tend to appear as slightly curved rods, showing varying irregularities in staining, as banding or beading, and in particular the presence at either end of small, deeply staining dots (metachromatic granules).

These granules may be seen in an eighteen-hour culture, but are more abundant after thirty-six hours. The granules are well seen with Löffler's blue, but better with Neisser's method. In culture the bacilli show swelling at one or both ends or clubbing. In secretions or in culture they show V-shapes or false branching and, what is most characteristic, the parallelism—four or five bacilli lying side by side like palisades. Being a Gram-positive organism while the majority of the other pathogenic bacilli are Gram-negative, it is of greatest importance to stain smears by this method. It is not so strongly tenacious of the gentian violet as the cocci, so decolorization should not be carried too far.

The best medium for growing it is Löffler's blood-serum.

An egg medium, made of the whole egg with glucose bouillon as described previously, is as suitable as Löffler's serum. Coagulated white of egg answers fairly well, as will a hard-boiled egg—the shell at one end being cracked and the white cut with a sterile knife. This smooth side is then inoculated and the egg placed cut side downward in a sherry glass. If an incubator is not at hand a tube may be carried next the body in a pocket. The bacillus grows better on glycerine agar than on plain agar. On such plates they appear as small, coarsely granular colonies with a central dark area. In size the colonies resemble the streptococcus. On blood-serum the colonies are larger—$\frac{1}{12}$ to $\frac{1}{8}$ inch in diameter.

The diphtheria bacillus grows luxuriantly on blood agar. In bouillon it tends to form a surface growth. It is at the surface that the toxin function is most marked, hence in growing diphtheria for toxin formation we use Fernbach flasks which expose a large surface to the air. It is a marked acid producer—bouillon with a +1 reaction becoming +2.5 to +3 in thirty-six hours.

Toxin and Toxone.—The filtrate from a two or three weeks' old broth culture is highly toxic, and is usually referred to as diphtheria toxin. It is used in injecting horses to produce antitoxin. Ehrlich used as a standard to measure the toxicity of toxin the minimal lethal dose (M. L. D.). This is the amount of toxin which will kill a 250-gram guinea-pig in just four days. Some toxins have been produced whose M. L. D. was $\frac{1}{500}$ c.c. or 0.002 so that 1 c.c. of such toxin would kill 500 guinea-pigs. Theoretically, the measure of an antitoxin unit is the capacity of neutralizating 200 units of a pure toxin. (On exposure to light, etc., toxin loses its toxic power and is termed toxoid.)

Almost invariably a bouillon filtrate contains toxones and toxoids besides the toxin. For all practical purposes it is usual to consider an antitoxin unit as that amount which will neutralize 100 M. L. D. (theoretically 200 units). Thus it would be the amount which would neutralize 0.2 c.c. of the above noted toxin. In testing filtrates as to their toxicity we make use of two limits, one designated Lo and the other L+. When we add increasing amounts of a filtrate containing toxins and toxones (the toxoids are less important because they do not show either the local reaction or palsy of toxones and do not possess the acute death-producing power of the toxin; they do, however, have combining power for antitoxin and are complicating factors in standardization) to an antitoxin unit we gradually reach a point where the slightest further increase will bring about slight reaction at site of inoculation of the guinea-pig and possibly some slight paralysis. These symptoms are due to toxone action. The amount of the toxic broth filtrate which is completely neutralized by one antitoxin unit is called Lo. Upon further addition of the filtrate to this Lo amount we finally reach a point when death of the 250-gram guinea-pig occurs in four days. This is called the L+ or fatal dose. Instead of having to add only that amount of filtrate which is capable of killing the guinea-pig (1 M. L. D.),

it is found that we must add an amount sufficient to kill 10 to 20 pigs. The explanation is that while toxin and toxone both have power to combine with antitoxin and to be neutralized, toxin has greater affinity and can dispossess toxone of its attachment. Consequently when all the combining strength of the antitoxin unit (equal to 100 M. L. D.) has neutralized the toxins and toxones added to it there is a complete blocking of injurious action of toxins or toxones. When adding more filtrate to the fixed amount of antitoxin a toxin molecule dispossesses a toxone molecule of its hold on the antitoxin unit. The toxin is neutralized but a toxone is put in circulation and is capable of causing reaction and palsy. Not until every toxone molecule has been displaced can the further addition of 1 M. L. D., containing sufficient toxin to kill, be free from the neutralizing antitoxin and capable of producing death in a 250-gram guinea-pig in four days. There have been prepared at the Hygienic Laboratory in the U. S. and in various European laboratories, by laborious testing of antitoxic serum, standardized antitoxin. By keeping dried antitoxin serum in a vacuum tube under conditions preventing exposure to heat, light and moisture the strength of the antitoxin unit remains stable. It may be stated that standardized toxin soon tends to change in potency, therefore it is customary to take exactly 1 unit of antitoxin and by adding to it increasing amounts of toxin to determine the amount which will be sufficient to kill the guinea-pig in four days. This is usually somewhat over the amount which will kill 100 guinea-pigs, or 100 M. L. D., and is designated the L+ dose of toxin. In practical application at biological product institutions the L+, dose is tested with increasing amounts of antitoxic serum, as drawn from the immunized horse, and that amount of serum which when mixed with the L+ dose of toxin just allows death of the guinea-pig in four days is accepted as one antitoxin unit.

In the preparation of antitoxin horses are employed; the method being to inject the bouillon filtrate or toxin subcutaneously at weekly intervals for a period of three or four months. When each c.c. of the serum of the horse is found to contain about 250 to 500 antitoxin units the horse is bled from the jugular vein. Some sera contain as much as 1300 units in a cubic centimeter.

Methods of purifying and concentrating antitoxin are now employed by certain makers, the principle being that the antitoxin in the horse serum is precipitated with the globulins which come down on half saturation with ammonium sulphate. In this way, as the content in horse-serum proteids is lessened, the anaphylactic dangers are lessened.

Dosage.—As a curative measure, from 2500 to 5000 units should be injected. If the injection is delayed or the case very serious the dose should be 10,000 units. As much as 50,000 units has been given in severe cases. The prophylactic dose is 500 units.

Schick Reaction.—By the employment of this reaction we can onderstand why one child develops clinical diphtheria and another unly shows the organism in the throat (laboratory diphtheria). We

find that certain persons have sufficient amount of diphtheria antitoxin normally in the circulation to protect against the soluble toxin elaborated by the organisms localized in throat or nose. Such cases show either a minimal or negative reaction.

Persons not having any antitoxin in the circulation show a positive reaction. The test is performed as follows: With a small sharp hypodermic needle we inject intradermally $\frac{1}{50}$ of a minimum lethal dose (1 M. L. D.) of diphtheria toxin as determined for a 250-gram guinea-pig. The standardized toxin is so diluted with a $\frac{1}{2}$% carbolic acid solution that 0.1 c.c. contains $\frac{1}{50}$ of a M. L. D. A positive reaction shows within twenty-four hours, reaching its maximum intensity in two days, as a reddened area, about 1 inch in diameter with more or less induration.

The reaction persists for about a week, leaving a brownish pigmentation. Positive reactions show that the patient has less than $\frac{1}{30}$ of a unit of antitoxin in 1 c.c. of his blood-serum and that he possesses no immunity to diphtheria.

This test is of great value as showing the cases needing prophylactic injections of antitoxin. Furthermore nurses showing a positive reaction should not take care of diphtheria patients. Carriers of true diphtheria usually show a negative reaction as contrasted with pseudo-diphtheria ones.

It is of value in showing duration and degree of immunity following antitoxin injections and such investigations have shown that intravenous injections are the most efficient, next the intramuscular and least efficient the subcutaneous route. Moody obtained an average of 45.2% positives in 524 people examined.

Immunization against Diphtheria.—It is well known that the prophylactic injection of diphtheria antitoxin gives but a short immunity—not more than 10 days. Where an epidemic is present or possibly in connection with persons not showing immunity by the Schick test one can give a more lasting (active) immunity by treating the individual with the diphtheria toxin-antitoxin mixture. In this $\frac{8}{10}$ of one L+ dose of toxin is overneutralized by one unit of diphtheria antitoxin and the serum so prepared is injected in 1 c.c. quantities subcutaneously. Three injections at intervals of about five days are required for immunization. The toxin-antitoxin mixture can be bought ready for injection. In an epidemic one could first give to those showing Schick reactions an immediate prophylactic injection of antitoxin to be followed by 3 injections at five-day intervals of the toxin-antitoxin immunizing mixture. Immunity is acquired in about 2 months.

Laboratory Diagnosis.—In obtaining material from a throat, be sure that an antiseptic gargle has not been used just prior to taking the throat swab. The part of the swab which touched the membrane or suspicious spot should come in contact

with the serum slant. This is best accomplished by revolving the swab. An immediate diagnosis is possible in probably 35% of cases by making a smear from a piece of membrane. In doing this Neisser's stain or the toluidin blue stain are usually considered the most satisfactory. I prefer the Gram stain, however. The diphtheria bacilli found in such smears are not apt to be clubbed and stain more uniformly.

If there is any doubt about the nature of an organism in a throat culture, always stain: 1. with Löffler's alkaline methylene blue for two minutes; 2. with Gram's method, being careful not to carry the decolorization too far, and 3. by Neisser's method. With Löffler's you obtain a picture which, after a little experience, is characteristic; at times the polar bodies show as intense blue spots in the lighter blue bacillus. One is liable to confuse cocci lying side by side for diphtheria bacilli with segmental or banded staining. This difficulty is not apparent when Gram's staining is used. This gives us great information, as the diphtheria and the pseudo-diphtheria are the only small Gram-positive bacilli usually found in the mouth. The cocci are also well brought out. Neisser's stain gives a picture which, when satisfactory, is almost absolutely characteristic. You have the bright blue dots lying at either end of the light brownish-yellow rods. When first isolated from a throat, the diphtheria bacillus is apt to stain characteristically by Neisser. Later on, in subculture, there may be no staining of the polar bodies. Neisser originally recommended five seconds' application, with an intermediate washing, for each of his two solutions. Thirty seconds for each is probably preferable. Some authorities recommend five to thirty minutes. It is well to bear in mind that about 2% of the people in apparent health carry diphtheria bacilli of the granular or barred type in their throats and of these about one in five will prove virulent for the guinea-pig.

It is essential when a question exists as to the nature of a diphtheria-like organism to test it as to virulence. While there are exceptions, especially in freshly isolated colonies, yet as a rule a severe infection yields virulent organisms and *vice versa*. Pure cultures are best obtained by streaking material from the throat on glycerine agar plates. From an isolated colony inoculate a tube of bouillon. From such a forty-eight- or seventy-two-hour-old culture inoculate a guinea-pig with 2 or 3 drops subcutaneously in the shaven abdomen. Escherich considers a fatal result with 1.5 c.c. of such a bouillon culture a satisfactory test as to virulence. After death, which occurs in two or three days, the adrenals are enlarged and hæmorrhagic.

Kolmer & Moshage recommend that a pure culture of the organism to be tested be grown on a slant of Loeffler's blood serum, washed off with 10 c.c. of sterile salt solution and 4 c.c. of the suspension be injected subcutaneously in the median abdominal line of 250–300 gram guinea-pig.

The diagnosis is more sure if, in addition to the first animal, a second one, which has had antitoxin, is inoculated. The protected one should live.

Diphtheroid Bacilli. Pseudodiphtheria Bacillus. Hofmann's Bacillus.—Under these terms various Gram-positive bacilli have been described as occurring in genito-urinary, nasal and skin diseases.

Their chief importance is in connection with their presence in the throats of healthy people. Probably approximately 10% of people harbor such organisms as against 1 to 2% with granule types. Some authorities believe it possible for these diphtheroids to be capable of being transformed into virulent diphtheria bacilli. This seems improbable. Such organisms are often found in urethral discharges, either alone, or with gonococci or other organisms.

Recently a great deal of attention has been given to the etiological relationship between diphtheroids and Hodgkin's disease. Fox, in a critical study of this relationship, has obtained diphtheroids of varying morphology and cultural characteristics from such glands as well as from enlarged glands of chronic atrophic arthritis and other conditions. It would appear conservative to reject the acceptance of diphtheroids as causative agents not only in this disease but in leprosy as well.

Negri has applied the name *Corynebacterium granulatis maligni* to diphtheroids isolated from glands in Hodgkin's disease. The granular rods of Much, supposed to be connected with tubercle bacilli, may be diphtheroids. Mallory has connected diphtheroids with scarlet fever.

DIPHTHEROID CHARACTERISTICS

1. They very rarely give the blue dot staining at the two ends. Exceptionally they may give a dot at one end. Neisser attaches importance to the dots at both ends as showing diphtheria.

2. They tend to stain solidly or at most with only a single unstained segment. They are shorter, thicker, and do not curve so gracefully as the true diphtheria bacillus. They are stockier.

3. They produce very little acid in sugar media, not one-half that produced by true diphtheria. Goldberger found 29 out of 30 cultures of *B. diphtheriæ* virulent and acid producers. Of 47 Hofmann cultures 6 showed slight acid production while 41 produced alkali. All were nonvirulent.

4. They are nonpathogenic for guinea-pigs.

5. Many of them grow quite luxuriantly and often show chromogenic power.

Xerosis Bacillus.—This organism is frequently found in normal conjunctival discharges. There is question as to its pathogenesis, and the finding of this organism should not exclude the previous presence of strictly pathogenic organisms, such as the *Gonococcus* or the Koch-Weeks.

It resembles the diphtheria bacillus in being Gram-positive and showing parallelism, but differs 1. in being nonvirulent for guinea-pigs; 2. in requiring about two days for the appearance of colonies; 3. in not showing Neisser's granule staining, and 4. in producing very little acid in sugar media.

CHAPTER VIII

STUDY AND IDENTIFICATION OF BACTERIA. GRAM-NEGATIVE BACILLI. KEY AND NOTES

KEY to the recognition of nonspore-bearing, nonchromogenic, non-Gram-staining, nonbranching bacilli.

(NOTE.—Some books say that the proteus group is Gram-positive. It is, however, usually negative.)

Do not grow on ordinary media. Require blood agar (hæmophilic bacteria), serum agar, or blood-serum.

Minute dewdrop colonies.

1. Influenza bacillus. Requires blood media.
2. Koch-Weeks bacillus (conjunctivitis). Serum agar best medium. Many, however, regard hæmoglobin as necessary for growth.
3. Müller's bacillus of trachoma. Like Koch-Weeks bacillus, but easier to cultivate.
4. Morax diplobacillus of conjunctivitis. Grows well and produces little pits of liquefaction in Löffler's blood-serum.
5. Bordet-Gengou bacillus of whooping-cough. Does not grow on Löffler's serum. Requires blood or ascitic fluid agar. Original isolation should be on glycerine potato agar.
6. Ducrey's bacillus (soft chancre). Requires media rich in blood or serum. Forms chains.

Grow well on ordinary media.

I. Cultures in litmus milk. PINK.

A. Nonmotile.

Lactis aerogenes group. B. lactis aerogenes.

Produce gas in glucose, lactose, and saccharose. No liquefaction of gelatin. Short, stubby bacteria, often showing capsules. Intermediate between the colon and Friedländer group.

B. Motile.

1. Nonliquefaction of gelatin.
   (*a*) B. coli group. Coagulation of milk. No subsequent peptonization. Gas in glucose and lactose, none in saccharose. Indol produced. Neutral red reduced.
2. Liquefaction of gelatin.
   (*a*) B. cloacæ group. Gas in glucose, slight in lactose. Slow coagulation of milk. Subsequent peptonization.

II. Cultures in litmus milk. LILAC.

A. Nonmotile bacilli.

1. No gas generated in glucose or lactose bouillon.

(*a*) Hæmorrhagic septicæmia group. These are oval bacilli with tendency to bipolar staining.

Colonies smaller and less opaque than those of B. coli.

Examples: B. pestis, B. suisepticus, B. choleræ gallinarum (chicken cholera).

B. pseudotuberculosis rodentium (very similar to plague).

B. pestis is absolutely nonmotile, does not liquefy gelatin, does not produce indol, produces slight acid in glucose but not in lactose bouillon.

(*b*) Dysentery group. Colonies similar to those of B. coli.

Divided into two classes according as mannite is acted on:

Those not giving acid—nonacid group—(Shiga-Kruse).

Those giving acid—acid group—(Flexner-Strong).

2. Gas generated in glucose bouillon not in lactose.

(*a*) Friedländer group. Give very viscid, porcelain-like colonies.

Tendency to capsule formation in favorable media.

Examples: B. pneumoniæ, B. capsulatus mucosus, B. rhinoscleromatis.

B. Motile bacilli.

1. Do not liquefy gelatin.

(*a*) Do not produce gas in either glucose or lactose bouillon.

Typhoid, or Eberth group. No indol. No coagulation of milk. No reduction of neutral red.

(*b*) Gas generated in glucose, not in lactose media. Milk not coagulated. Neutral red reduced.

Gärtner group. This includes:

Pathogenic types for man; as B. enteritidis, B. icteroides, B. paratyphoid B, B. psittacosis. Nonpathogenic for man; as B. choleræ suum (hog cholera).

2. Liquefy gelatin.

(*a*) Proteus group. Colonies at first round, later amœboid, spreading. Produce gas in glucose, not in lactose. Produces foul odor.

B. zopfii type of Proteus group does not liquefy gelatin; colonies at first round, later amœboid, spreading. Foul odor in cultures. Gelatin stab shows lateral branching.

NOTE.—The Friedländer and the lactis aerogenes group differ culturally chiefly in carbohydrate fermentation activities and organisms considered as belonging to the Friedländer group rather than to the lactis aerogenes group may show acid in litmus milk. Where an organism having the characteristics of *B. coli*, but fermenting saccharose, is found, it is termed *B. coli communior*. A non-gas producing colon type organism has been designated *B. coli anaerogenes*. Certain organisms which turn litmus milk lilac and which liquefy gelatin, but do not produce gas in sugar media, belong to the "Booker" group. Other organisms which acidify and coagulate litmus milk but do not liquefy gelatin or produce gas in glucose or lactose

media have been placed in the "Bienstock" group. The proteus or "Hauser" group is composed of organisms showing various functions; *Proteus vulgaris* liquefying gelatin rapidly, *P. mirabilis* slowly and *P. zenkeri* not at all. The differentiation of the colon group is extensively considered under bacteriology of water.

Gram-negative Bacilli Requiring Special Media

Bacillus Influenzæ (Pfeiffer, 1892).—This organism is the type of the so-called hæmophilic bacteria—organisms whose growth is restricted to media containing hæmoglobin. The influenza bacillus seems to grow better on slants freshly streaked with blood than on those which have been made for some time, and they appear to grow better on this surface smear of blood than on a mixture of agar and blood. The blood agar plate is very convenient for the isolation of *B. influenzæ*.

The influenza bacilli are most likely to be isolated from the sputum of bronchopneumonia due to this organism. It has also frequently been found in the nasal secretions of influenza patients. Exceptionally, it is present in the blood, and has been isolated in cases of meningitis from cerebrospinal fluid. It also occurs at times in anginas, but then usually associated with other organisms. Infection probably only takes place by contact. It is a very small bacillus which in sputum tends to show itself in aggregations, especially centering about *M. tetragenus*. It stains rather faintly when compared with cocci, so that a smear of sputum stained with formol fuchsin shows a deep violet staining for the *M. tetragenus* or other cocci, and scattered around in a clump-like aggregation we see these minute, rather faintly stained rods. They also tend to stain more deeply at either end, so that they sometimes appear as diplococci. Gram's method, counterstaining with formol fuchsin is excellent for their demonstration. The red bacilli and the violet-black cocci are easily distinguished.

To cultivate them, rub the sputum, or at autopsy the material from a lung, on a slant smeared with human blood (pigeon's blood is also satisfactory), and then without sterilizing the loop, inoculate a second blood slant; then a third, and possibly a fourth.

The colonies appear as very minute dewdrop-like points which seem to run into each other in a wave-like way. To test such colonies we should transfer a single colony to plain agar and blood-serum, trying not to carry over any blood. If the least trace of blood is carried over, they may grow on agar or blood-serum. Organisms resembling the influenza bacillus have been isolated from whooping-cough. Such organisms have also been found in the fauces of well persons. In many epidemics of influenza the bacillus has not been isolated, or success has obtained in only a small proportion of the cases. Etiological factors in conditions more or less resembling influenza may be the *Streptococcus*, *Pneumococcus*, or *M. catarrhalis*. The influenza bacillus seems to grow best in symbiosis with some other organism, especially with *S. pyogenes aureus*. The influenza bacillus has, as a rule, very slight

virulence for experimental animals although rarely a strain may be encountered which is virulent for guinea-pigs.

An attack of influenza does not seem to give immunity. Not infrequently influenza involves the lungs and the organism may cause a septicæmia, otitis, meningitis or even conjunctivitis and pyelitis.

Consumptives are liable to become carriers of the influenza bacillus, the organism being present in their sputum and such patients may not only themselves suffer from repeated attacks of influenza but may infect others.

Koch-Weeks Bacillus (Koch, 1883).—This produces a severe conjunctivitis. It is very common in Egypt and is also a frequent cause of conjunctivitis in the Philippines and in temperate climates.

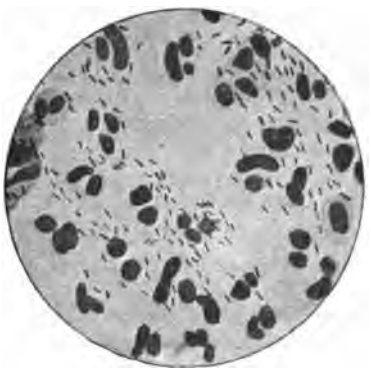


FIG. 31.—The Koch-Weeks Bacillus. (*Hansell and Sweet.*)

Smears made from conjunctival secretion show large numbers of small Gram-negative bacilli, especially contained within pus cells, but also lying free. They are more difficult to cultivate than the influenza bacillus, but the same general methods hold. The vitality of this organism is very slight so that almost immediate transference of material is necessary. Flies are an important factor in Egypt. The period of incubation is short, twelve to thirty-six hours. The best medium is a mixture of glycerine agar and hydrocele or ascites fluid. At first we rarely obtain pure cultures. The colonies are dewdrop-like and first show themselves in about thirty-six hours in incubator cultures.

It would seem that blood agar is a better medium than a serum one. Many hæmoglobinophilic bacteria will grow with only 1 to 500 hæmoglobin, so that growth on serum might be explained by slight blood admixture.

Some have thought that repeated infection with the Koch-Week's bacillus was the cause of trachoma. Others have regarded other hæmoglobinophilic bacteria as

causative. According to the views of Park and Williams the inclusion bodies of Prowazek, supposed to be characteristic of trachoma, are simply clumps of extremely small, coccoid hæmoglobinophilic bacteria. Besides these organisms the *Gonococcus* in smears from gonorrhœal ophthalmia is stated to show involution forms, making a resemblance to trachoma bodies.

Noguchi thinks that while there is a morphological similarity between degenerated hæmoglobinophilic bacteria and cell inclusions, yet in the latter, the elementary bodies are much smaller than the bacterial granules, and the initial bodies less definite in contour. He was able to infect the conjunctivæ of monkeys with inclusion bodies material, but not with hæmoglobinophilic bacilli.

Diplobacillus of Morax.—This organism causes mild blepharo-conjunctivitis chiefly at the internal angle of the eye. They are about 1 or 2μ long and tend to occur in pairs or short chains. Some claim that they are Gram-positive.

Culturally the formation of little pits of liquefaction in Löffler's serum within twenty-four hours which later become confluent may be regarded as fairly characteristic. They do not grow on nutrient agar.

After two or three days on blood-serum rather marked involution forms occur. While usually causing a more or less chronic conjunctivitis they may at times produce a keratitis.

NOTE.—A Gram-negative bacillus which is less than 1 micron long, growing singly, or in pairs, and known as the bacillus of Zur Nedden has been stated to produce corneal ulcers. It grows readily on agar or other ordinary culture media. It coagulates milk.

Bacillus of Chancroid (Ducrey, 1889).—These are short coccobacilli, occurring chiefly in chains. They show bipolar staining. They grow best in a mixture of blood and bouillon.

Material for culturing should be obtained before the lesion ulcerates. The exudate should be inoculated on blood agar, 1 part blood to 2 of agar. After forty-eight hours small glistening colonies develop which easily slide about the slant when touched with a loop.

Bacillus of Bordet-Gengou.—This bacillus was reported as the cause of whooping-cough by Bordet and Gengou in 1906. (Czaplewski and Reyher had previously reported oval bipolar staining organisms, as the cause of pertussis, and other authors influenza-like organisms.)

The bacillus is oval, Gram-negative, shows bipolar staining, somewhat resembles *B. influenzæ* and grows only on uncoagulated serum media, as blood or ascites agar.

The original cultures are very scanty so that the colonies are difficult to recognize. In subcultures the growth is more flourishing. The organism is only found in white, thick, leukocyte abounding sputum, of the beginning of the disease. Hence pertussis is probably contagious only at the onset.

Complement binding and agglutination reactions have been obtained. For diagnosis stain the sputum. Remember that pertussis gives a mononuclear leukocytosis of 15,000 to 50,000.

For isolation from sputum the following medium is required. Autoclave 500 grams potato with 1000 c.c. of 4% glycerine solution. Pour off excess of fluid. Emulsify potato in 1500 c.c. normal salt solution and add powdered agar to 3 or 4%. For use mix with an equal quantity of defibrinated blood.

It is not entirely settled that *B. pertussis* is the cause of whooping-cough. It must be remembered that in culturing sputum from a case we may obtain a Gram-negative bacillus, which, however, shows a profuse growth even on ordinary agar.

Gram-negative Bacilli Growing on Ordinary Media

Bacillus Pneumoniæ (Friedländer, 1882).—This organism is responsible for about 5% of the cases of pneumonia. It is usually termed the *Pneumobacillus* to distinguish it from the *Pneumococcus;* at other times Friedländer's bacillus. The name of Fraenkel attaches to the *Pneumococcus*. Morphologically, it is a short, thick bacillus, and in pathological material, as sputum, shows a wide capsule. It is nonmotile and Gram-negative. The colonies on agar are of a pearly whiteness and are markedly viscid. On potato it shows a thick viscid growth containing gas bubbles. The characteristic culture is the nail culture of a gelatin stab. The growth at the surface is heaped up like a round-headed nail, the line of puncture resembling the shaft of the nail.

It does not liquefy gelatin. It does not produce indol, and does not produce gas in lactose bouillon—differences from the colon bacillus—with which it may be confused in cultures, as it does not then possess a capsule. If in doubt, inject a mouse at the root of the tail. Death from septicæmia occurs in two days. The peritoneum is sticky and numerous capsulated bacilli are present in the blood and organs. The organisms which have been isolated from rhinoscleroma and ozœna are practically identical with the *B. pneumoniæ*. This group of organisms is generally referred to as the Friedländer group. Similar organisms have been isolated from the discharges of middle-ear diseases and in anginas. Cases have been reported where the *B. pneumoniæ* was the cause of septicæmia in man. The organism has been reported from cases of both lobar and lobular pneumonias, but such pneumonias are rare and, when occurring, of a fatal tendency.

Bacillus Pestis (Kitasato, Yersin, 1894).—This is the organism of plague. It is primarily a disease of rats. It is the member of the group of hæmorrhagic septicæmias (Pasteurelloses), from which man suffers.

Other Pasteurelloses are chicken cholera, swine plague, mouse septicæmia and rabbit septicæmia. This is a widely distributed group and may include saprophytic organisms as well as those noted for their virulence.

B. choleræ gallinarum and *B. suisepticus* are approximately similar in size and cultural requirements to *B. pestis*. The oval bacillus with bipolar staining in smears from tissues is very characteristic for both of them. Another name for swine plague (*B. suisepticus*) is infectious

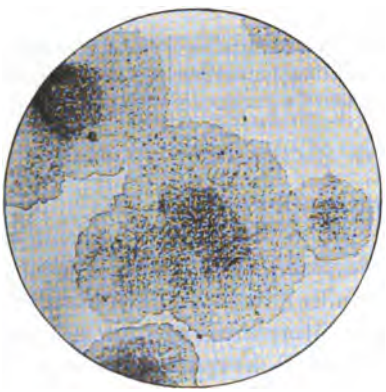


FIG. 32.—Colonies of plague bacilli forty-eight hours old. (*Kolle and Wassermann.*)

pneumonia of swine. The organism is chiefly found in the lungs. The bacillus of plague was first isolated by Yersin from a plague bubo, in 1894, at Hong Kong. It is true that Kitasato reported a bacillus which he had isolated from the blood of a plague patient, on July 7, 1894 (Yersin's report was made July 30, 1894). Kitasato's bacillus was motile, Gram-positive, coagulated milk and gave a turbidity in bouillon, characteristics which were just the opposite of those of the organism reported by Yersin.

Where the plague bacilli are found chiefly in the glands, we have bubonic plague; when in lungs, pneumonic plague; when localized in the skin and subcutaneous tissue, the cellulo-cutaneous; and when as a septicæmia, septicæmic plague. An intestinal type is recognized by some authors. It must be remembered that in all forms of plague the lymphatic glands show hemorrhagic œdema; it is in bubonic plague, however, that the areas of necrosis with periglandular œdema are prominent.

Types.—Where the symptoms are slight, mainly buboes, the term pestis minor is sometimes used; the typical disease being termed pestis major. In pneumonic plague we have a bronchopneumonia.

In smears from material from buboes, from sputum, or in blood smears, as well as from blood or spleen smears from experimental animals, we obtain the typical morphology of a coccobacillus ($1.5 \times 0.5\mu$) with very characteristic bipolar staining; there being an intermediate, unstained area. Very characteristic also is the appearance in these smears of degenerate types which stain feebly and show coccoid and inflated oval forms. The presence of these involution forms associated with typical bacilli is almost diagnostic for one with experience. Inoculating tubes of plain agar and 3% salt agar with this same material, we obtain in plain agar culture organisms which are typically small, fairly slender rods, which do not stain characteristically

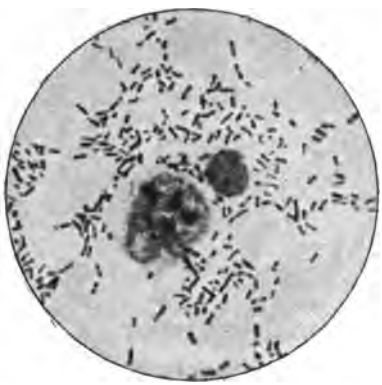


FIG. 33.—Pest bacilli from spleen of a rat. (*Kolle and Wassermann.*)

at each end and are not oval. The smear obtained from the salt agar presents most remarkable involution forms—coccoid, root-shaped, sausage-shaped forms, ranging from 3 to 12 microns in length, more resembling cultures of moulds than bacteria. Another point is that on the inoculated plain agar we are in doubt at the end of twenty-four hours whether the dewdrop-like colonies are really bacterial colonies or only condensation particles. By the second day, however, these colonies have an opaque grayish appearance, so that now, instead of questioning the presence of a culture, we consider the possibility of contamination. Litmus milk is rendered slightly acid but not sufficiently to change the lilac color. Glucose broth is made slightly acid but there is no effect on lactose.

Blood cultures in septicæmic plague may show from 5 to 500,000 bacilli per c.c. Smears from the blood in such cases are positive in only about 17%.

The plague bacillus grows well at room temperature—its optimum temperature being 30° instead of 37°C., as is usual with pathogens.

Next to the salt agar culture, the most characteristic one is the stalactite growth in bouillon containing oil drops on its surface. The culture grows downward from the undersurface of the oil drops as a powdery thread. These are very fragile, and as the slighest jar breaks them, it is difficult to obtain this cultural characteristic.

Confusing Organisms.—While Klein states that *B. coli*, *Proteus vulgaris* and, in particular, *B. bristolensis* may be mistaken for plague bacilli, if bipolar staining alone be relied upon, yet it is *B. pseudotuberculosis rodentium* which may confuse an experienced worker. While this latter is only moderately pathogenic for rats yet the fact that rats may be immunized to *B. pestis* by inoculation with *B. pseudotuberculosis*

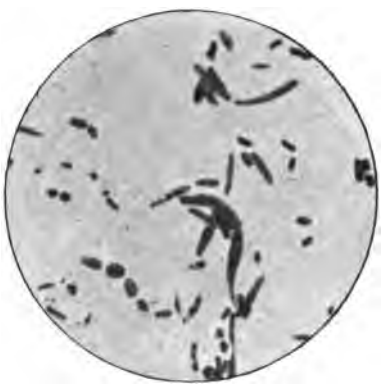


FIG. 34.—Pest bacillus involution forms produced by growing on 3% salt agar. (*Kolle and Wassermann.*)

rodentium brings up the suspicion of identity of the two organisms. In diagnosing always use animal experimentation. Owing to the difficulty in emulsifying plague bacilli, agglutination tests are not satisfactory. *B. tularense* resembles *B. pestis*. *B. tularense* produces a conjunctivitis. See ocular infections, Part IV.

The Crucial Test.—Albrecht and Ghon have shown that by smearing material upon the intact, shaven skin of a guinea-pig, infection occurs. This is the crucial test.

A pocket made by cutting the skin of a guinea-pig with scissors and extended subcutaneously with scissors or forceps, into which a piece of the suspected plague tissue is thrust with forceps, is more practical than injecting an emulsion with hypodermic syringe.

Animal Plague.—Mice inoculated at the root of the tail quickly succumb. Rats, this being primarily a disease of rats, are of course susceptible. Other rodents, as squirrels, are susceptible. It has been suggested that a rodent, the Siberian marmot,

or tarabagan (*Arctomys bobac*) might be the starting-point of plague outbreaks. In natural plague of rats, the lesions which establish a diagnosis even without the aid of a microscope are dark red, subcutaneous injection of the flaps of the abdominal walls as they are turned back, fluid in the pleural cavities, œdematous hæmorrhagic periglandular infiltration and swelling of the neck glands, and in particular a creamy, mottled appearance of the liver.

Danysz Virus.—The bacillus known as Danysz virus also causes whitish granules of liver but these are larger and do not have the appearance as if peppered on the liver.

The neck glands in rat plague are chiefly involved because the flea prefers to inhabit the skin of the neck. The spleen is swollen, congested and granular and smears from this viscus will show the bacilli.

A chronic rat plague, which may be a factor in keeping up the disease, is characterized by enlargement of the spleen and the presence within it of nodules containing plague bacilli. McCoy has noted that the frequency of the cervical bubo in rats, noted by the Indian Commission (72%), was not found in California. The glands show periglandular infiltration and injection as well as enlargement.

Transmission.—Recent investigations in India have definitely determined the fact that the flea (*Xenopsylla cheopis*) is the intermediary in the transmission of plague from rat to rat and from rat to man.

In Europe and U. S., *Ceratophyllus fasciatus* is the common rat flea and it, as well as other species of flea, may transmit the disease. The bedbug will also transmit plague. Fleas suck up the septicæmic blood of infected rats and there is a development of plague bacilli in the œsophagus with more or less obstruction. When feeding on man or other rats such fleas regurgitate and thus inoculate these plague bacilli. The fæces of such fleas are also infectious.

Pneumonic Plague.—In primary pneumonic plague the infective nature is very great and appears to be by the respiratory atrium (from man to man). This was the terrifying type of plague in the black death of the fourteenth century.

Strong and Teague have shown that of 39 plates exposed before the mouths of patients with pneumonic plague, with marked dyspnœa and pulmonary œdema, but without coughing, only 1 plate showed plague bacilli. In 39 other experimental plate cultures with coughing on the part of the patients there were 15 plates showing plague bacilli.

The droplet method of infection is therefore the important one in plague pneumonia.

As these droplets are expelled to a considerable distance not only should the respiratory inlets be protected by masks but the conjunctivæ with glasses and abrasions with protective coatings.

For diagnosis make smears and cultures from material drawn from a bubo by a syringe. (At a later stage, when softening begins, there may not be any bacilli present.) Also, if pneumonic plague, from the sputum. Blood cultures and even blood smears may be employed in septicæmic plague. Formol fuchsin and Archibald's stain make satisfactory stains. Always inoculate a guinea-pig with the material either by rubbing it in with a glass spatula on the shaven skin or by subcutaneous injection.

For prophylaxis the most important method is that of Haffkine. Stalactite bouillon cultures of plague are grown for five to six weeks. These are killed by a temperature of 65°C. for one hour. Lysol (¼%) is added to the preparation and from 0.5 to 4 c.c. injected, according to the age and size of the individual treated. Susceptibility is reduced about one-fourth, and of those attacked after previous vaccination, the mortality is only about one-fourth of what it is among the noninoculated. Strong prepares a prophylactic vaccine from living plague cultures rendered avirulent. Yersin's serum, made by injecting horses with dead plague cultures and afterward with living ones, is of value prophylactically and has possibly considerable curative power.

The Eberth, Gärtner and Escherich Groups

From a standpoint of cultures in litmus milk and sugar bouillon we can divide the organisms related to typhoid at one extreme and the colon at the other into three groups.

1. **The Eberth or Typhoid Group.**—There are three important pathogens in this group: the *B. typhosus*, the *B. dysenteriæ*, and the *B. fæcalis alkaligenes*. The color of litmus milk is practically unaltered and there is no gas production in either glucose or lactose bouillon. No coagulation of milk. No reduction of neutral red. The *B. typhosus* and the *B. fæcalis alkaligenes* are actively motile, while the *B. dysenteriæ* is nonmotile or practically so.

During the first twenty-four to forty-eight hours there is a moderate acid production by typhoid, so that the milk culture is less blue, while with the *B. fæcalis alkaligenes* the alkalinity is intensified from the start, so that the blue color is deepened

2. **The Gärtner or Hog Cholera Group.**—Besides organisms important for animals and probably at times for man, such as *B. choleræ suum* and *B. psittacosis* and *B. icteroides* (interesting historically as having been reported as the cause of yellow fever by Sanarelli), we have three pathogens: 1. *B. enteritidis* (Gärtner's bacillus); 2. *B.*

paratyphoid A. and 3. *B. paratyphoid B.* In this connection it may be stated that the present view is that hog cholera is caused by an ultra-microscopic organism and not by the *B. choleræ suum.*

These organisms cannot be separated culturally, but only by immunity reactions. They do not turn litmus milk pink. They produce gas in glucose bouillon, but not in lactose. They very powerfully reduce neutral red with the production of a yellowish fluorescence. They do not coagulate milk. There is a transient acidity in the litmus milk, but becoming shortly afterward alkaline, the lilac-blue color is intensified. These organisms are motile.

3. **The Escherich or Colon Group.**—These turn litmus milk pink, coagulate milk, reduce neutral red, and show varying degrees of motility.

The three groups of organisms just described are nonliquefiers of gelatin. Two intestinal organisms, the *B. cloacæ* and the *Proteus vulgaris*, differ in liquefying gelatin.

Bacillus Typhosus (Eberth, 1880; Gaffky, 1884).—This organism may be isolated from the stools, urine, and the blood of typhoid patients.

At postmortem it can be best isolated from the spleen, but is also present in Peyer's patches which have not ulcerated. When ulceration has occurred contamination with *B. coli*, is almost sure. Cultures may be obtained from the liver also. In sections made from spleen the Gram-negative bacilli are apt to be decolorized. Thionin, then blotting and clearing in oil or xylol, shows the clumps of bacilli lying between the cells.

Formerly it was supposed that by the differences in the thickness of the film of a colony or by its varying shades of grayish-blue, we possessed data of importance in differentiating typhoid from related organisms.

The colonies look like grapevine leaves.

Growth on potato was also considered as affording information. At present, the biochemical reactions give us information assisting in differentiation, and the agglutination and bacteriolytic phenomena, the final diagnosis. The various plating media are considered under media for plating out fæces.

Not only do we find hyperplasia of the endothelial cells in the lymphoid tissue of Peyer's patches and the mesenteric glands and the spleen, with subsequent necroses, but focal necroses of the same character are found in the liver.

Pathology.—A striking feature of the pathology of typhoid fever is the long-continued persistence of the organisms in the gall-bladder and elsewhere.

It is beginning to be believed that a previous typhoid infection, possibly so mild as to have passed unnoticed, is at the basis of gall-bladder infections and resulting

gall-stones. Various bone infections, especially osteomyelitis, have shown the typhoid bacilli in pure culture. Formerly it was supposed that the typhoid bacillus brought about its lesions by a local infection centered in the ileum.

The present view is that typhoid bacilli effect an entrance into the blood stream through some lymphoid channel, as by tonsil or other alimentary lymphoid structure.

Of animals, only the chimpanzee seems to be susceptible.

They develop in the general lymphatic system, the spleen in particular, where they are protected from the bactericidal power of the blood.

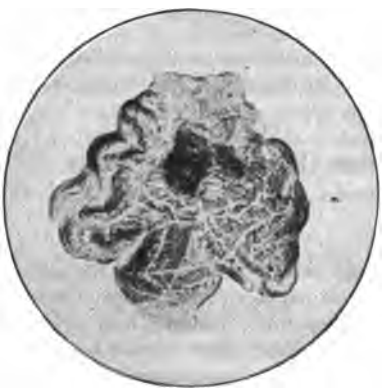


FIG. 35.—Seventy-two-hour-old culture of typhoid bacillus on gelatin. (*Kolle and Wassermann.*)

After a time, however, approximately the period of incubation, they become so abundant in these lymphatic organs that they are carried over into the general circulation. Then as a result of bacteriolysis the intracellular toxins are liberated and symptoms develop. If bacteriolysis takes place other than in the blood we have various suppurative processes. As a result of the formation of antibodies, the development in spleen, etc., is checked but should these immunity reactions become less potent relapses may occur or various local infections manifest themselves.

As the bacilli do not multiply to any extent in the blood itself the disease cannot be considered as a typical septicæmia but as a bacteriæmia.

Animals are not susceptible to typhoid fever with the possible exception of the higher apes. Of course the injection of living or dead cultures may kill an animal but there are no characteristic localizing symptoms.

The Blood.—Typhoid bacilli can be isolated from the blood during the latter period of incubation and rarely after the tenth day of the disease. It is a practical point that the time to isolate the bacteria from the blood is in the first days of the attack. The diagnosis by agglutination is only expected after the seventh to tenth day. Agglutination may not appear until during convalescence, and in about 5% of the cases it is absent. It, as a rule, disappears within a year.

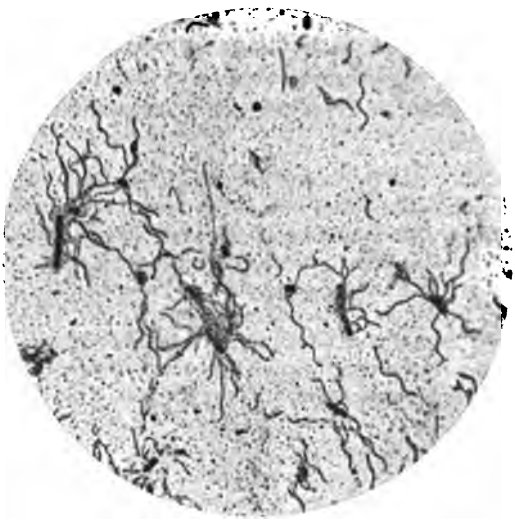


FIG. 36.—Bacillus of typhoid fever, stained by Löffler's method to show flagella. (×1000.) (*Williams.*)

Typhoid bacilli may be found not only in the blood, urine and fæces but as well in the sputum of cases showing pulmonary involvement. They have also been found in the cerebrospinal fluid of cases showing meningeal symptoms. At the autopsy they may be found in the spleen, Pyer's patches, mesenteric glands and liver.

Very little success has been obtained with curative sera. Chantamesse, by treating horses with a filtrate from cultures of typhoid bacilli on splenic pulp and human defibrinated blood, claimed to have obtained a curative serum possessing antitoxic power.

Prophylactic Vaccination Against Typhoid.—Wright's method of prophylactic inoculation is now being employed in the British army with apparent success. In this, twenty-four- to forty-eight-hour-old cultures are killed at 53°C.; ¼% of lysol is then added. An injection of 500,000,000 bacteria is made at the first inoculation, and ten days later an injection of 1,000,000,000. The British prefer to inject subcutaneously in the infraclavicular region and at the insertion of the deltoid. The Germans consider 3 injections as conferring greater immunity.

Russell has obtained splendid results in the U. S. Army with his method of vaccination. In this 3 injections are given at intervals of ten days, the dosage being 500,000,000, for the first and 1,000,000,000 for each of the 2 succeeding injections.

Typhoid vaccines sterilized with 0.5% of phenol appear to keep much longer and to have a higher immunizing power than those prepared by sterilization with heat and subsequent addition of the antiseptic.

On account of paratyphoid fevers appearing in the forces on the Mexican border vaccination with paratyphoid bacilli was instituted, but separate from the typhoid one.

At present all men in the military services are vaccinated with a *triple vaccine* made up of 1,000,000,000 typhoid bacilli, 750,000,000 paratyphoid A bacilli and 750,000,000 paratyphoid B organisms in 1 c.c. of the vaccine.

The first dose is 0.5 c.c., the second and third 1 c.c. each. The triple vaccine is inoculated subcutaneously at intervals of one week. Craig has carefully studied the reactions following the inoculations of the triple vaccine and could observe no more severe reactions than occur with the typhoid vaccine alone. A temperature rise (rarely above 101°F.) following inoculation is noted in about 32% following the first injection, 86% after the second and 18% after the third. About one-half of those inoculated complain of headache after the first and second inoculations but rarely experience it following the third injection. Malaise is frequently noted.

When tested for agglutinins, one month from the time of the first inoculation, the serum agglutinates typhoid bacilli in from 1–640 to 1–1260 on the average. For paratyphoid A a slightly higher effect and somewhat less so for paratyphoid B. The English obtained higher titres for paratyphoid B than for paratyphoid A. It is usually considered that immunity for typhoid lasts about two years and a degree of protection even after four or five years. With the paratyphoids the immunity is thought to be of less duration. In times of trench warfare it is probably advisable to repeat the inoculations yearly, but ordinarily this may be delayed for three years. For typhoid-paratyphoid agglutination see Dreyer technic.

Carriers.—A very important discovery is that certain persons, who may have had only a slight febrile attack, may eliminate typhoid bacilli for years in their faces (typhoid carriers). The bacilli are also eliminated for considerable periods in the urine. Distinction is now being made between acute carriers (convalescents) and chronic carriers.

In experiments on higher apes there was evidence that the bacilli eliminated by carriers are in many instances nonpathogenic. About one-half of typhoid cases are believed to be due to contact infections. Drigalski gives it for Germany as 64.7%. The water transmission factor is of less importance than was formerly stated.

The most satisfactory method of detecting carriers is by examination of fæces or urine plated out on Endo's medium. While carriers usually give a Widal reaction this is by no means constant. Typhoid carriers are said to maintain a high opsonic index.

The urine and fæces of typhoid convalescents should be proven negative by cultural procedure before discharging the patients.

Various methods have been tried to cure these carriers, such as vaccination and even cholecystectomy but without any particular evidence of success.

For laboratory diagnosis, blood cultures during the first week and agglutination tests during the second week and onward are the practical methods.

Along with the agglutination tests the urine and fæces should be cultured on Endo's plating medium and later transferred to Russell's medium for cultural identification. The positive identification, provided the culture so isolated shows the cultural characteristics of typhoid, is made by testing the bacilli for agglutination with a known typhoid serum. Instead of the usual blood cultures one may use the clot in the Wright U-tube for culturing and the serum remaining after centrifugalization for the Widal test (*clot culture*). *B. typhosus* appears in the blood in relapses.

Contact infection is the great factor in perpetuating typhoid fever but this agency shows diminishing cases each year provided water and milk supplies are safe. The leading European cities as a result of a safe water supply rarely show more than about 3 typhoid deaths per 100,000 population per year. Edinburgh shows less than one per 200,000 for the year 1910. In American cities rates of 12 to 15 per 100,000 are common.

Kayser considered that about 27 % of cases of typhoid in Strasburg were caused by raw milk, 17 % by contaminated water, 17 % by contact with typhoid, and 10 % were due to typhoid carriers. Other cases were due to infected food, and about 13 % were of origin impossible to determine. These latter may have been due to unrecognized typhoid carriers. He does not attach the same importance to fly dissemination as do American authors.

The Gärtner or Meat-poisoning Group.—Under this designation may be considered the organisms which cause gastrointestinal disorders of varying degrees, infection with which is usually brought about by the ingestion of meat obtained from diseased cattle. Unless the meat is thoroughly cooked the bacilli in the interior may not be killed.

In this group may be placed *B. enteritidis*, the typical meat-poisoning organism, *B. paratyphoid B*, *B. Danysz*, *B. Aertryck*, *B. typhi murium* and *B. suipestifer*.

B. suipestifer or the hog cholera bacillus was formally thought to be the cause of this important epizootic. It is found in the intestines of quite a percentage of healthy hogs. The cause is now known to be a filterable virus.

These organisms are alike morphologically and culturally and show quite a tendency to bipolar staining and reduction of neutral red with fluorescence in forty-eight hours. *B. paratyphoid B*, *B. Aertryck* and *B. suipestifer* are alike from an agglutination standpoint, while *B. enteritidis* and *B. Danysz* show similarity in this respect. *B. paratyphoid A* stands by itself.

Paratyphoid Bacilli (Achard and Bensaude, 1896; Schottmüller, 1901).—Cases resembling mild attacks of typhoid occasionally show agglutination for paratyphoid bacilli. These organisms have also been isolated from the blood, as with typhoid. Two types have been recognized: the paratyphoid A and the paratyphoid B. The latter occurs in 80% of such cases. Culturally, paratyphoid B cannot be separated from Gärtner's bacillus. In paratyphoid A there is less gas produced in glucose bouillon than with paratyphoid B, and the primary acidity of litmus milk is not succeeded by a subsequent alkalinity. It does not seem practical to draw a fine distinction between these two strains.

Krumwiede attaches importance to acid production in xylose by all members of the Gärtner group, except *B. paratyphoid A*.

The brownish discoloration on lead acetate agar by *B. paratyphoid B* is also a differentiating point.

The same points as to transmission, diagnosis and carrier state noted under typhoid hold for the paratyphoids.

The symptoms of paratyphoid A infections are rather like those of typhoid but generally milder. Cases have been reported where paratyphoid A even showed the intestinal lesions of typhoid. Various local infections with the paratyphoids, especially pyelitis, have been noted. Some cases of infectious jaundice have been attributed to paratyphoid infection.

Paratyphoid B not only gives symptoms resembling a mild typhoid infection, but may show symptoms more like those of meat poisoning or even cholerine. It is more pathogenic for laboratory animals than is *B. typhosus*. The development of antibodies upon immunizing a man or animal with paratyphoid organisms does not seem to approach that obtained with typhoid.

Castellani has conducted experiments with typhoid and paratyphoid vaccines and has found that typhoid vaccines give an agglutinating serum of about 1 to 350 titre from the second to fifth week dropping to about 1 to 100 after three or four months. Paratyphoid A gives one of about 1 to 75 for the first month which drops to about 1 to 60 after four months. Paratyphoid B gives about one-half the agglutination response that paratyphoid A does. These results do not correspond with those of Craig noted under typhoid vaccination.

Bacillus Enteritidis (Gärtner, 1888).—This organism has been frequently isolated from cases of gastroenteritis from ingestion of infected meat.

Meat from healthy animals which has been in contact with that of diseased animals may become infected. The simple act of placing a piece of infected meta

on a sound piece may infect the latter. It has been noted that the bacteria, or their toxins, may be distributed unevenly in the meat eaten, so that one person consuming the same meat may be made very ill while others eating this meat may escape infection. Infection of food may occur not only from unclean handling but from the material carried by flies or even from the fæces of mice or rats deposited on foodstuffs.

This organism is very pathogenic for laboratory animals, producing a hæmorrhagic enteritis and at times a septicæmia. Where meat has been contaminated with Gärtner's bacillus toxins may have been produced, and symptoms of poisoning with acute gastroenteritis would occur shortly after ingestion. This is not a true toxin as it does not require a period of incubation befo e manifesting its toxic action. It is interesting to note that this toxin is not destroyed by the boiling temperature, thus differing from the toxin of the other important meat-poisoning (botulism) bacillus—*B. botulinus*—which is rendered innocuous by a temperature of 65° or 70°C. If there is only a little toxin introduced with the contaminated meat, the symptoms will be delayed one or two days. Such organisms have been isolated in pure culture from cases with high fever, marked intestinal derangement, with considerable blood in the rather fluid stools. In two cases studied the disease was at first diagnosed as a severe typhoid infection. Klein thinks the organism of Danysz's virus (to kill rats during plague epidemics) may be identical with *B. enteritidis*.

Proteus Vulgaris.—This organism is often encountered in plates made from fæces, or sewage contaminated water.

It is common in decaying meat or cheese, and cases of even fatal poisoning with marked gastrointestinal symptoms and cardiac failure have been reported. Such poisoning may have been due to ptomaines elaborated by the proteolytic action of the organism on the food, rather than from *B. proteus* itself. At times it is the cause of cystitis. The colonies on agar are moist and unevenly spreading (amœboid). The bacilli are very motile, long and slender, tend to form filaments and, as a rule, are Gram-negative. It digests blood-serum and is a rapid liquefier of gelatin. In litmus milk it coagulates with a soft clot and an alkaline reaction. Subsequently the litmus is reduced and the clot digested giving a dirty yellowish-brown fluid. Indol is rarely produced. The cultures generally have a putrefactive odor. In infective jaundice (Weil's disease) this organism has been reported as the cause. Organisms of this group were formerly designated as *B. termo*.

Bacillus Dysenteriæ (Shiga, 1898).—Dysentery bacilli produce a coagulation necrosis of the mucous membrane of the large intestine and occasionally of the lower part of the ileum. Polymorphonuclears are contained in the fibrin exudate.

It was formerly thought that these lesions were of local origin, but the present view is that toxins are produced which, being absorbed, are eliminated by the large intestine with resulting necrosis. Flexner, by injecting rabbits intravenously with

a toxic autolysate, produced characteristic intestinal lesions. The toxin withstands a temperature of 70°C. without being destroyed. The toxin may cause joint trouble.

There are two main types of dysentery bacilli:

1. Those producing acid in mannite media—the acid strains (Flexner-Strong types).

2. Those not developing acid in mannite (Shiga-Kruse types). Ohno finds that fermentative reactions do not correspond to immunity ones. Thus an acid strain used to immunize a horse may produce a serum more specific for a nonacid strain. Hiss, however, found that organisms similar in fermentation reactions agreed in agglutination ones. The Shiga type is very toxic in cultures, possibly having a soluble toxin, while the Flexner type does not seem to possess a soluble toxin.

Clinically the toxæmia of cases of dysentery due to Shiga types is marked while that from Flexner strains is slight.

We often designate the Shiga strains as the toxic group and the acid-producing strains as the non-toxic. The "Y" type of organisms also produces acid in mannite. The following is the Lentz table:

Lentz recognizes 4 types of dysentery bacilli for the differentiation of which he uses mannite, maltose and saccharose bouillon with litmus as an indicator.

	Shiga-Kruse	Flexner	Strong	"Y"
Mannite	Blue	Red	Red	Red
Maltose	Blue	Red	Blue	Blue
Saccharose	Blue	Blue	Red	Blue

A strain which ferments not only mannite, dextrose, maltose and saccharose, but dextrin as well, is known as the Harris type.

The Shiga strains are apt to cause a paresis of the hind extremities of the injected rabbit which may be followed by paralysis and death. At the Lister Institute injections of a soluble toxin produced a serum of marked antitoxic power. Such a dysentery serum, which is probably both antitoxic and antimicrobic, is of curative value. Shiga immunized horses with polyvalent cultures and obtained a polyvalent serum which has reduced the death rate about one-third.

Dysenteric Stools.—The dysentery bacillus is present in the milky white, leukocyte-filled, blood-flecked mucous stools during the first five or six days of the disease. By the tenth day it has probably disappeared. Lactose litmus agar is the most satisfactory plating medium.

The stool of the first two days may give practically a pure culture. The staining of a smear from the muco-purulent stool is rich in phagocytic cells, many of them packed with Gram-negative bacilli. In all cultural respects the dysentery bacillus resembles the typhoid, and the only practical method of distinguishing these two organisms, other than by agglutination reactions, is by the nonmotility or exceedingly slight motility of the dysentery bacillus.

The characteristic of nonmotility is of greatest differentiating value and the reports of slight motility are probably from misinterpretation of molecular movement as motility. The dysentery bacilli do not form those threads or whip-like filaments so characteristic of typhoid cultures and are somewhat plumper.

As a rule they occur singly or in pairs, and of rather oval or even coccoid shape and may stain bipolarly. The colonies are much like those of typhoid. Type "Y" colonies often show indentation while the Shiga types show round colonies.

The dysentery bacillus is not found in the blood and hence is not eliminated in the urine. It is found in mesenteric glands. In dysentery patients agglutination phenomena do not show themselves until about the twelfth day from the onset. Hence, this procedure is of no particular value in diagnosis. It is of value, however, to identify an organism isolated from the stools at the commencement of the attack, using serum from an immunized animal or a human convalescent for the agglutination test.

Agglutination.—Butler has suggested taking serum from dysentery convalescents, noting the strain involved, and preserving it by taking up with filter-paper as recommended by Noguchi for the Wassermann hæmolytic amboceptor. This I consider very valuable as it is very difficult to immunize rabbits with a Shiga strain on account of its great toxicity. Dean has reduced the toxicity of Shiga vaccines by treating with eusol.

There seems to be very little agglutination power in the serum of convalescents from Shiga strains. Flexner strains give agglutination, but early in convalescence the serum is not apt to have a titre of more than 1 to 50.

Ritchie has recently tested the sera of 792 normal persons and found that 30% of these individuals agglutinated Shiga bacilli in 1 to 32, while with Flexner strains 41% agglutinated in 1 to 64 and 30% in 1 to 128. For comparison Ritchie's results with typhoid showed that only 6% agglutinated such bacilli in 1 to 16. There is some evidence that typhoid vaccination increases the agglutinating power of the serum against dysentery organisms. These findings are remarkable, as the usual advice is to consider an agglutination of 1 to 30 as fairly specific for Shiga infections and 1 to 100 for Flexner ones.

Lipovaccines.—Whitmore has recently reported most favorable results from using a new method for preparing dysentery vaccines. The growth from the culture flasks (Kolle) is removed with a vacuum scraper which obviates the necessity

for addition of fluid to remove the growth. This growth is collected in Petri dishes, frozen and dried *in vacuo*. The dried bacterial mass is then incorporated in olive oil, which has been sterilized in the autoclave at 15 pounds for fifteen minutes. The bacterial growth is ground for forty-eight hours in large glass stoppered bottles with steel ball bearing balls. Then in sufficient lanolin to make up 10% of the completed vaccine in the olive oil. The oil suspension is then heated at 53°C. for one hour. The vaccine contains 3 billion Shiga organisms, about the same content of "Y" organisms and about 2 billion Flexner type bacilli in 1 c.c. of the lipovaccine. Such a vaccine gives rise to no more general reaction than does the triple typhoid one in saline.

B. Morgan, No. 1.—Morgan has reported as the cause of certain cases of bacillary dysentery a bacillus known as *B. Morgan, No.* 1. It is motile, produces indol, and in glucose bouillon gives a very slight amount of gas.

It does not change mannite and does not produce a primary acidity in litmus milk. This organism is a frequent cause in England of summer diarrhœa of children. Flies from houses with such cases often show Morgan's bacillus. A dysentery type much like the Flexner-Strong strain is often found in the enteric affections of children in the United States.

Ekiri.—In Japan, dysentery-like epidemics of a very fatal disease, termed *ekiri*, occur among young children. The organism is very motile, producing gas and acid in glucose but not in lactose media. It is reported at times to show indol production. Apparently a member of the Gärtner group.

More recently a strain of dysentery bacilli, known as *Type Y*, has been considered of importance. This organism is very closely related to the Flexner strain and only differs from it in that it requires about forty-eight hours to turn mannite litmus media pink and that maltose litmus remains blue. An organism showing similar cultural characteristics has been recently recovered from fæces of laboratory rabbits by German workers investigating the problem of whether certain animals might serve as carriers for dysentery.

B. Coli, B. Lactis Aerogenes, B. Cloacæ

While the COLON BACILLUS chiefly inhabits the large intestine, the *B. lactis aerogenes* is to be found in the upper part of the small intestine. While they may be separated on the ground of motility, yet it is by the greater fermentative activity of the *B. lactis aerogenes* that they are best separated. Some consider them as only representing different strains of the same organism.

The main points of distinction are gas production in starch media (gas bubbles on potato slant) and frequent nonproduction of indol. *B. lactis aerogenes* is closely related to the pneumobacillus and at times shows capsules. It is best differentiated from the pneumobacillus by its gas production in lactose and coagulation of milk.

Some consider that the *B. coli* produces a bactericidal substance which inhibits the growth of, or destroys, pathogenic bacteria which may have passed the destructive influences of the gastric juice; others that this effect is due to their free growth and the development of phenol and various putrefactive substances.

The probable importance of the colon bacillus in protecting the organism is shown by the fact that where numerous colonies of pathogenic organisms may be cultivated from fæces we may find a diminution in number or absence of the colon bacillus. This condition may be observed in infections with the organisms of dysentery, cholera, typhoid and paratyphoid. While its normal function is probably protective, yet the *B. coli* is an important pathogenic agent, it being frequently the organism isolated from purulent conditions within the abdominal cavity, especially in appendicitis and lesions about the bile ducts. It is particularly prone to cause lesions of the bladder and pelvis of the kidney. In the treatment of colon cystitis by vaccines of dead colon bacilli, brilliant results in opsonic therapy have been obtained.

Sir A. Wright thinks that certain cases of mucous colitis may be due to colon infection and that vaccination may cure them. The colon bacillus is fully considered under the bacteriology of water.

B. cloacæ was isolated first from sewage by Jordan. It is, as a rule, a rapid liquefier of gelatin, and in its reactions with sugars and litmus milk resembles the colon bacillus.

Where the gelatin liquefaction is slow or slight *B. cloacæ* may be distinguished from *B. coli* by its gas formula which is about three times as much CO_2 as H, just the reverse of that of the colon bacillus. *B. lactis aerogenes* is also often found in sewage. It is one of the causes of the souring of milk.

B. Acidophilus, B. Bifidus, B. Bulgaricus

These are often termed the long rod group of lactic acid bacteria in contradistinction to certain other Gram-positive bacilli which are short and oval and which are confused with the so-called milk streptococci.

The long rod group often forms chains and often shows metachromatic granules which stain with Neisser's method. They are readily distinguished from Gram-negative lactic acid producers, of which the type is *B. lactis aerogenes*, by their Gram-positive staining. *B. acidophilus* often give the impression of a diphtheroid

in a Gram-stained fæces smear. It is nonmotile and often shows polar granules. Grows only at temperatures above 22°C., op. 37°C. It grows better anaerobically than aerobically and then shows the clubbed involution characteristics of *B. bifidus;* so that some consider these organisms the same, the morphology of *B. bifidus* being the result of anaerobiosis. Original cultures are best made in 1% glucose and 1% acetic acid bouillon. Some authorities consider *B. bifidus* the most important representative of the large intestine flora. *B. lactis acidi* is less thermophilic than *B. acidophilus* and coagulates milk which *B. acidophilus* does not do. Certain polar granule bacteria, as *B. granulosum,* found in Yoghurt, are similar to *B. acidophilus* but coagulate milk; no gas. *B. bulgaricus* is the type of the group and is discussed under milk.

Rodella thinks *B. acidophilus, B. bifidus, B. gastrophilus* and the Boas-Oppler bacillus identical. *B. bulgaricus* is said to never show polar granules. *B. bulgaricus* and the group of organisms similar to it found in buttermilk, etc., are widely used in the treatment of various intestinal troubles. North has used cultures of *B. bulgaricus* for extermination of undesirable organisms in other parts of the body than the alimentary canal (used as applications in nasal, throat or genito-urinary infections).

Chromogenic Bacilli

These are identified by the color of their colonies on agar. The *B. pyocyaneus* is the most important one of them in medicine, but the *B. prodigiosus* is also of interest medically. A violet chromogen, the *B. violaceus,* which is motile and liquefies gelatin, has been described under many names. It has been found in water.

An orange-yellow chromogen, the *B. fulvus,* is nonmotile and varies as to its liquefaction of gelatin.

B. Pyocyaneus (Gessard, 1882).—This organism is frequently termed the bacillus of green or blue pus. It is a small ($2.5 \times 0.5\mu$) motile Gram-negative bacillus.

It is generally a slender delicate bacillus often showing thread-like arrangement but at times it may appear as short plump rods. It grows readily at room or incubator temperature. It liquefies gelatin rapidly. The green color diffuses through the agar or gelatin on which it grows, so that we not only have the green-colored colony, but the medium as well is colored. Upon potato the colonies are more of a deep olive green to dirty brown.

No gas is produced in either glucose or lactose bouillon; blood-serum is digested, the pitted surface showing a reddish-brown color. The protein ferment pyocyanase has been used to remove diphtheritic membrane and for treatment of *M. catarrhalis* nasal catarrhs. There are 2 pigments—a green water soluble one and a blue one soluble in chloroform.

It is widely distributed in water and air, and is frequently isolated from fæces. The *B. fluorescens liquefaciens* of water seems to be simply a strain of *B. pyocyaneus*. The *B. pyocyaneus* is frequently associated with other pus organisms in abdominal abscesses.

In addition to having an endotoxin, it produces a soluble toxin similar to diphtheria toxin. This toxin differs from those of diphtheria and tetanus in that it can stand a temperature of 100°C., while those of diphtheria and tetanus are destroyed at about 65°C. The fact that the union between toxin and antitoxin is only of a binding, neutralizing nature is best shown by taking a mixture of pyocyaneus toxin and antitoxin which is innocuous and heating it. This destroys the antitoxin, but does not injure the toxin. We now find that the original toxicity has returned. The antitoxins of diphtheria and tetanus are more stable than the corresponding toxins; hence, this experiment would be impossible with them, as upon heating we should first destroy the toxin.

FIG. 37.—*Bacillus pyocyaneus.* (*Kolle and Wassermann.*)

On account of the frequent association of *B. pyocyaneus* with other organisms of better recognized pathogenicity it has until more recently been considered rather harmless; this view can no longer be entertained as it is frequently the sole cause of middle-ear inflammations, intestinal disorders, cystitis and possibly at times of septicæmia.

B. Prodigiosus.—This is a very small coccobacillus which shows motility in young bouillon cultures. It is Gram-negative. The colonies on agar or other solid media show a rich red color. The pigment only develops at room temperature, it is absent in cultures taken out of the incubator. The *B. prodigiosus* is frequently found on foodstuffs, especially bread, where it may simulate blood. It liquefies gelatin rapidly and gives a diffuse turbidity to bouillon. It is probable that *B. indicus* and *B. kilensis* are strains of *B. prodigiosus*.

Coley's fluid, which has been used in cases of inoperable sarcoma and other malignant growths, is a culture prepared by growing very virulent streptococci in bouillon for ten days. This *Streptococcus* culture is now inoculated with *B. prodigiosus*, and after another ten days the mixed culture is killed by heat at 60°C. and the sterile product injected. Coley injected about $\frac{1}{20}$ c.c. of this vaccine. At present he uses nonfiltered, heat sterilized bouillon cultures of a *Streptococcus* obtained either from a case of erysipelas or septicæmia. To this is added material from agar cultures of *B. prodigiosus*, grown separately and sterilized before adding to the sterilized streptococcus bouillon culture.

CHAPTER IX

STUDY AND IDENTIFICATION OF BACTERIA. SPIRILLA. KEY AND NOTES

KEY to recognition of gelatin liquefying, motile and Gram-negative spiral or comma-shaped organisms.

A. Do not give the nitroso-indol reaction with sulphuric acid alone in twenty-four hours.

1. Produce an abundant moist cream-colored growth on potato at room temperature.
   (*a*) Finkler and Prior's spirillum (Vibrio proteus). Liquefaction of gelatin very rapid. No air-bubble appearance at top of liquefied area. Cultures have foul odor. Milk coagulated. Thicker spirillum than cholera. Isolated from cholera nostras.
2. Scanty growth or none at all on potato at room temperature. Only a moderate yellowish growth when incubated about incubator temperature.
   (*a*) Spirillum tyrogenum (Deneke's spirillum). Does not liquefy gelatin so rapidly as Finkler Prior. Thinner and smaller spirillum than cholera.

B. Give the nitroso-indol reaction with sulphuric acid within twenty-four hours.

1. Very pathogenic for pigeons.
   (*a*) Spirillum metschnikovi. Liquefies gelatin about twice as rapidly as cholera. Gives bubble appearance at top of stab.
2. Scarcely pathogenic for pigeons.
   (*a*) Spirillum choleræ asiaticæ.

Nonmotile, nonliquefying and Gram-positive spirilla have also been described. There is also a large group of phosphorescent spirilla.

Spirillum Choleræ Asiaticæ (Koch, 1884).—Typically, the morphology of this organism is that of the comma (Comma bacillus of Koch). It also frequently shows S shapes, and often appears in long threads showing turns. When freshly isolated from cholera material they, as a rule, show a fairly typical morphology, but after subcultures in the laboratory variations are common, so that rod forms and round involution shapes give a picture altogether at variance with the comma shape.

Even in recent cultures of undoubted cholera we may have different types, as coccoid forms and slender rods. Ohno has noted the fact that the same strain of cholera will give at one time vibrio forms and again coccoid or rod forms, depending

on the reaction of the media. Inasmuch as the recognition of vibrio shapes is of importance in diagnosis he recommends that material from a stool be inoculated into 3 tubes of peptone solution of reaction +0.3, −0.5 and −1, respectively, one of which would probably show vibrio morphology.

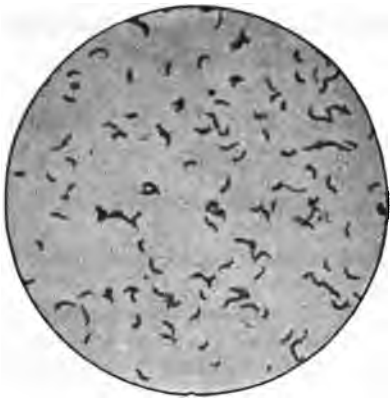


FIG. 38.—Cholera spirilla. (*Kolle and Wassermann.*)

The cholera spirillum is very motile (a scintillating motility) and liquefies gelatin fairly rapidly, although more slowly than any of the spirilla mentioned in the key.

FIG. 39.—Involution forms of the spirillum of cholera. (*Van Ermengen.*)

The colony on gelatin was formerly considered characteristic, but like most cultural characteristics, it is now considered as being only of confirmatory value; it is not specific. These colonies show in twenty-four hours as small granular white spots which have a spinose periphery. An encircling ring of liquefaction now makes its appearance and the highly refractile (as if fragments of sparkling glass) colony can be separated into a granular center, a striated periphery, and a clear external ring of liquefaction.

On gelatin stabs the liquefaction produces a turnip-like hollow at the top of the puncture—the air bubble appearance. It gives the nitroso-indol reaction with sulphuric acid alone (cholera red). Kraus attaches importance to the fact that cholera does not produce a hæmolytic ring on blood agar as do the pseudocholera spirilla; a difficulty is that many pseudospirilla do not hæmolize. Furthermore, true cholera strains may occasionally show hæmolysis, especially in laboratory cultures. Quite a discussion has arisen in connection with a spirillium isolated from cases of diarrhœa (no symptoms of cholera) in pilgrims at El Tor. This organism gave the immunity reactions (agglutination) of true cholera but on account of its hæmolytic power has been considered as distinct from cholera. Such a view would seem to be untenable. *Sp. choleræ* grows very rapidly on peptone solution and this is the medium for the enrichment test to be later described. On this it may form a pellicle. On agar the colony is more opalescent (more of a translucent grayish blue) than the typhoid. It does not grow on potato except at incubator temperature. It does not coagulate or turn acid litmus milk. Some strains, however, do produce a certain amount of acid. Using the Hiss serum sugar media our strains produced acid in glucose and saccharose but not in lactose. No gas production in any of the sugars. The spirilla are found in myriads in the rice-water discharges, these white flakes being desquamated epithelial cells. They penetrate the crypts of Lieberkuhn, but rarely extend to the submucosa. The symptoms are due to an endotoxin.

FIG. 40.—Spirillum of cholera stab culture in gelatin two days old. (*Fraenkel and Pfeiffer.*)

Cholera may be transmitted from water supplies, when the outbreak is apt to be widespread and in great numbers from the start. Also by indirect contagion, as by flies or on lettuce, etc. A very important point is that we have well persons whose fæces contain virulent cholera spirilla (cholera carriers).

Cholera spirilla disappear from the stools of cholera patients very rapidly, usually in five to ten days.

Only exceptionally are organisms excreted longer than three or four weeks, but cases are on record of periods approximating two or three months. Cholera carriers in good health may come down with cholera as the result of administration of purgatives or alimentary canal disorder. This would explain periods of incubation longer than the usual one of two to five days.

Cholera carriers are therefore of less importance epidemiologically than typhoid carriers, where the carrier stage may last years.

It is well to remember, however, that cases have been reported of positive findings after a period approximating two months from the onset of the attack of

cholera. Another important consideration is that the vibrios may be absent at one examination and be present at a later one. Purgatives seem to influence the reappearance of the spirilla. An acid reaction of the fæces, such as that induced by lactic acid bacteria, would apparently be of value in the prophylaxis of cholera carriers.

Greig has found infection of the bile of the gall-bladder or ducts in 80 cases in 271 cholera autopsies. While cholera spirilla are soon crowded out by intestinal bacteria, thus explaining the short period during which cholera spirilla are excreted by convalescents, this is not true when the cholera vibrio gets into the bile ducts or gall-bladder, where ideal conditions prevail for a prolonged life. In fact bile has recently been recommended as a selective medium for cholera enrichment. Greig found one cholera convalescent excreting cholera vibrios forty-four days after the attack. Of 27 persons who had been in contact with cholera patients 6 were excreting cholera vibrios though apparently well.

To identify such spirilla immunity reactions are necessary:

1. Injected intraperitoneally into guinea-pigs, it produces a peritonitis and subnormal temperature. This reaction exists for spirilla other than the true cholera spirillum.

2. Intramuscular injections into pigeons are only slightly pathogenic, if at all.

3. The agglutination test is the most practical. In this we use serum from an immunized animal, in dilution of from 100 to 1000. It is rare that true cholera vibrios fail to agglutinate in serum of 1 to 500 and even sera of 1 to 10,000 dilution give the reaction. Serum of cholera convalescents may show agglutination as early as the tenth day; it is usually best shown about the third week. Dunbar's quick method is very practical. Make two hanging-drop preparations, using mucus from the stool as the bacillary emulsion. To one add an equal amount of a 1 : 50 normal serum; to the other a 1 : 500 dilution of immune serum. Cholera spirilla remain motile in the control, but lose motility and become agglutinated in the preparation with the immune serum.

4. Pfeiffer's phenomenon. If cholera spirilla are introduced into the peritoneal cavity of immunized guinea-pigs (or if together with a 1 : 1000 dilution of immune serum the mixture is injected intraperitoneally into normal guinea-pigs) and at periods of ten to sixty minutes after injection, material is removed by a pipette from the peritoneal cavity, the spirilla have lost motility, have become granular and degenerated. Pseudospirilla are unchanged. This reaction may be carried on in a pipette, using fresh serum.

Antisera for the treatment of cholera have not proved successful.

On the whole the reports from the use of anticholera sera are not very encouraging. Savas, however, was favorably impressed by such treatment during the Balkan war. It should be administered intravenously and early in the attack and given in doses of 50 c.c. Of 61 severe cases so treated the mortality was 55.7%. Of 17 severe cases not receiving serum treatment all died.

Prophylactically, there are three prominent methods:

1. That of Haffkine, where live cholera spirilla are injected subcutaneously; and

2. Strong's cholera autolysate. In this cholera cultures are killed at 60°C. The killed culture is then allowed to digest itself in the incubator at 37°C. for three or four days (peptonization). The preparation is then filtered and from 2 to 5 c.c. of the filtrate is injected. Ferran was the first to use vaccines.

3. At present the same methods are being used for cholera vaccines as for those of typhoid. An emulsion of the vibrios in salt solution or a bouillon culture is subjected to a temperature of 54° C. for one hour. Three doses are injected seven to ten days apart, going from 500,000,000 to 2,000,000,000.

Among the Greek forces 0.45% of the inoculated and 1.9% of the noninoculated were attacked. The inoculated, however, were sanitary troops and hence more exposed to infection.

For diagnosis: 1. take a fleck of mucus, make a straight smear and fix; stain with a 1 : 10 carbol fuchsin. The comma-shaped organisms appear as fish swimming in a stream.

2. Inoculate a tube of peptone solution. The cholera spirilla grow so rapidly, and being strong aerobes, they grow on the surface of the fluid so that by taking a loopful from the surface, we may in three to eight hours obtain a pure culture. Should there be a pellicle present, this should be avoided in the transfer by tilting the tube slightly, so that the material near the surface be obtained without touching the pellicle. Inoculate a second tube from the surface of this first and, if necessary, a third (enrichment method).

3. Test for cholera red reaction. (Simply adding from 3 to 5 drops of concentrated chemically pure sulphuric acid to the first or second peptone culture after eighteen to twenty-four hours' growth. Some specimens of peptone do not give the reaction.) At times we only get the cholera red when we have a pure culture of cholera.

4. Smear a fleck of mucus or, better, the three-hour surface growth of a peptone culture on a dry agar surface in a Petri dish. From colonies developing, make agglutination and, if desired, cultural tests. It is by immunity reactions that we identify cholera spirilla. The surface moisture of plates is best dried by the filter-paper top.

The cholera colony is easily distinguished from the ordinary fæcal bacterial colonies by its transparent, bluish-gray, delicate character. It emulsifies with the greatest ease. A practical, quick method is to make smears from suspicious colonies, stain for one minute with dilute carbol fuchsin and if vibrios are present to make 2 vaseline rings on a single slide allowing ample space at one end for handling the preparation safely. Inside of one ring deposit with a platinum loop a drop of

salt solution and inside the ring nearest the end which is to be held by fingers or forceps, deposit a loopful of 1 to 500 or 1 to 1000 dilution of cholera serum. The emulsion in the salt solution remains uniformly turbid and under a low power of the microscope (⅔ inch) shows a scintillating motility. The emulsion made into the drop of serum quickly shows a curdy agglutination and upon examination with the ⅜-inch objective shows clumping and absence of motility. Cover-glasses placed over the 2 vaseline rings assist in the study of the preparation.

Cholera selective media are considered under "Culture Media."

CHAPTER X

STUDY AND IDENTIFICATION OF MOULDS

Classification of the Fungi

Order	Suborder	Family	Genus	Species
Phycomycetes	Zygomycetes	Mucoracidæ	Mucor	M. corymbifer
				M. mucedo
			Rhizomucor	R. parasiticus
			Rhizopus	R. niger
Ascomycetes	Gymnoascees	Saccharomycetidæ	Saccharomyces	S. cerevisiæ
				S. anginæ
				S. blanchardi
			Endomyces	E. vuillemini
			Cryptococcus	C. gilchristi
				C. hominis
		Gymnoacidæ	Trichophyton	T. sabouraudi
				T. tonsurans
				T. violaceum
				T. mentagrophytes
				T. cruris
			Microsporum	M. audouini
			Achorion	A. schoenleini
			Penicillium	P. montoyai
				P. crustaceum
			Sterigmatocystis	S. nidulans
	Carpoascees	Perisporiacidæ	Aspergillus	A. fumigatus
				A. concentricus
				A. pictor
				A. niger
			Discomyces	D. bovis
				D. maduræ
Hyphomycetes			Madurella	M. mycetomi
			Malassezia	M. furfur
			Microsporoides	M. minutissimus
			Monilia	M. albicans
			Trichosporum	T. giganteum
			Sporotrichum	S. beurmanni

Note.—In many of the works on bacteriology considerable space is given to the so-called Higher Bacteria. The organisms are chiefly considered under the names *Leptothrix* or forms in which are found simple nonbranching threads, *Cladothrix* or thread-like forms with false branching and *Streptothrix* or forms showing true branching. It is not practical to consider any separate group distinct from the so-called Lower Bacteria on the one hand and the Fungi on the other.

The Thallophyta are plants in which there is no differentiation between root and stem.

The classes of Thallophyta which are of interest medically are 1. the Algæ and 2. the Fungi.

The Algæ contain chlorophyll. An exception to this is with the Schizophyta, algæ which include the Bacteriaceæ or Schizomycetes and the Schizophyceæ or Cyanophyceæ. The bacteria do not contain chlorophyll and the Cyanophyceæ or blue-green algæ contain a blue pigment (phycocyanin) in addition to chlorophyll. It is in their relation to bacteria that algæ are important. Some authorities consider the family Bacteriaceæ as belonging to the order Cyanophyceæ.

Diseases caused by fungi are known as mycoses.

Some include Lichenes as a separate class. These are really symbiotic organisms—Fungi parasitic on Algæ.

The fungi do not possess chlorophyll. They are in their simplest forms ramifying filaments called hyphæ. The vegetative hyphæ which intertwine in tangled threads, as a support, are termed the mycelium, while those which project upward are called the aërial hyphæ and are the ones which bear the conidia or spores.

The aërial hypha which carries the fruiting organ encasing the conidia (sporangium) is called the sporangiophore and the more or less rounded termination of this hypha, which projects into the sporangium, is called the columella.

The hypha may be composed of one cell or of many cells separated by septa (septate).

The orders of the class Fungi which are of interest medically are: 1. the Phycomycetes; 2. the Ascomycetes; 3. the Hyphomycetes.

Phycomycetes.—These produce a copious network-like mycelium, which is nonseptate, and reproduce asexually by means of a sporangium, a case-like structure borne on the clubbed extremity of an erect hypha (columella) and containing numerous spores or, as in the case of the suborder Oomycetes, reproduction is by heterogamy. (Dissimilar sexual cells—a smaller male, antheridium, and a larger female, oogonium. By fertilization by antherozoids from the antheridium penetrating the oösphere we have oöspores.)

The suborder Zygomycetes reproduces either asexually (a sporangium filled with spores) or by isogamy (two similar but sexually differentiated cells conjugate and form on fusion a zygospore).

Belonging to this suborder we have four families, only one of which, the Mucoracidæ, is of importance medically. In this family we have three genera: *Mucor*, without rhizoids; *Rhizopus*, with rhizoids and unbranched aërial hyphæ and, *Rhizomucor*, with rhizoids and ramified mycelium.

Under anaerobic conditions the nonseptate mycelium of these fungi may break up into short septa resembling yeasts.

Two species of *Mucor* are of pathogenic importance.

1. *Mucor mucedo* and 2. *Mucor corymbifer*. These moulds develop especially in external cavities as nasopharynx and external ear.

Pulmonary and generalized infections have also been reported. The pathogenic species have smaller spores and grow best at 37°C. The thick, coarse, cotton-like mould seen on horse manure is a *Mucor*. The sporangium, the organ of fructification, contains the spores within its interior. The *M. mucedo* has thick silver-gray mycelium, with large sporangia, 150μ in diameter, containing oval spores, 5×9μ. The *M. corymbifer*, which has been reported from a generalized infection, considered as typhoid, shows a snow-white mycelium. The sporangia are 20 to 40μ and the spore about 3μ in diameter.

Rhizomucor parasiticus has been reported from the sputum of a woman with a condition resembling phthisis.

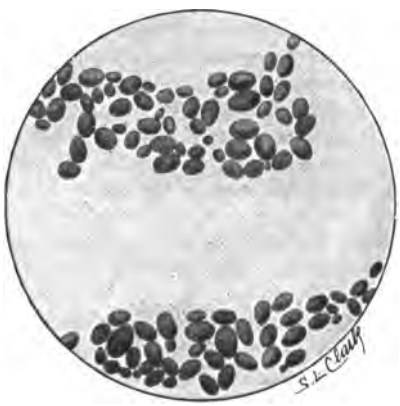


FIG. 41.—Yeast cells. *Saccharomyces cerevisiæ*. (*Coplin.*)

Rhizopus niger has a columella which becomes distorted into a mushroom shape after the spores have been discharged from the sporangium. This mould has been considered as the cause of a mycosis of the tongue.

Ascomycetes.—In this order are included many of the parasitic moulds. The most distinctive characteristic is the formation of ascospores in an ascus (little sac).

It is an enlarged extremity of a hyphal branch in which a definite number of spores, usually eight, is formed. The ascus usually ruptures at its tip. Other members of the order are formed from hyphæ by the separation of cells in succession from the free cells. The mycelium is septate.

The order is divided into those with naked asci (Gymnoascees) and those having a perithecium or investing layer about the ascus (Carpoascees).

Belonging to the suborder Gymnoascees we have 1. the family of Saccharomycetidæ, which reproduce by budding and in which the asci are without any semblance of a sheath, and 2. a family in which there is an indication of the formation of a perithecium—this may be termed the Gymnoascidæ family.

SACCHAROMYCETIDÆ.—There are three genera: *Saccharomyces*, *Endomyces*, and *Cryptococcus*.

Saccharomyces.—These reproduce by budding, have ascopores and no mycelial-like threads.

S. cerevisiæ.—This is the ordinary yeast fungus. Used at times as an antiseptic. It is also used in treatment of beriberi as it is very rich in vitamines.

S. anginæ.—Found in a case of angina.

S. blanchardi.—Found in a jelly-like tumor mass of the abdomen. The budding cells varied from 2 to 20μ. Probably identical with *S. tumefaciens*, reported as the cause of a subcutaneous tumor about region of Scarpa's triangle.

Endomyces.—Forms spores in the interior of filaments, or by ascus formation or by chlamydospores (resistant spore-like structures with a thick membrane which project from the extremities or sides of the hyphæ as bud-like structures).

E. vuillemini.—One of the organisms of thrush. It produces a false membrane, especially on buccal surfaces, which is easily detached and beneath which the mucosa is intact. Grows only in acid media. Hence propriety of alkaline treatment. Some authorities consider the genus *Endomyces* as the same as *Monilia* or *Oidium*.

Cryptococcus.—Reproduces by budding, but ascospore formation not observed. Not a well-recognized genus. The diseases caused by it are termed blastomycoses.

C. gilchristi.—The cells are about 16μ in diameter and have a thick, double contoured membrane. They reproduce by budding. The skin lesions resemble various infectious granulomata and diagnosis rests on the finding of budding or sporulating cells. It may invade internal organs. Original cultures are obtained with some difficulty and then best with Löffler's serum. Subcultures grow readily. Potato is a good medium and on it we may have both mycelial and yeast-like growth. Guinea-pigs can be inoculated subcutaneously. A mould, somewhat similar, is the *Coccidioides immitis* of Ophuls. This has a mycelial growth in tissues, this distinguishing it from the former fungus. The large cells frequently show yeast-like bodies within, hence the characteristic of endogenous spore formation. The large round encapsulated cells of *C. gilchristi* or *C. hominis* do not show contained spores. The infection frequently becomes generalized. The small bodies, about 3μ, in the *Molluscum contagiosum* cells are thought by some to be yeasts. They are more probably artefacts. Plimmer's bodies in cancer cells belong in this group. They also are probably other than parasites.

C. linguæ pilosæ.—This is a more or less elongated yeast-like organism and supposed to be the cause of black tongue, a benign affection of the lingual papillæ.

GYMNOASCIDÆ.—Belonging to the family Gymnoascidæ we have the genera *Trichophyton*, *Microsporum*, *Achorion*, *Endodermophyton* and *Epidermophyton*.

Trichophyton.—The fungi of the genus *Trichophyton* are generally known as the large-spored ringworms. The spores are in chains and may be inside the hair or both outside and inside. Many of them are of animal origin, especially from the horse and the cat. The spores are from 5 to 15μ.

The mycelium is greatly segmented, shows simple or dichotomous branching, and produces spores within the mycelium.

T. tonsurans.—Gives a crater-like cuture with fine marginal rays. Fungus wholly inside the hair. Causes most of the large-spored scalp ringworms and many body cases.

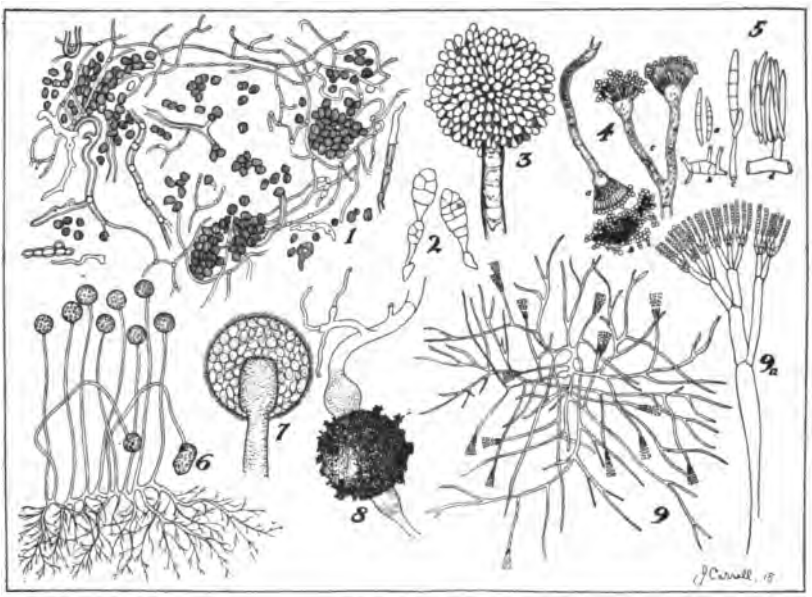


FIG. 42.—1, Tinea versicolor scrapings (*Malassezia furfur*). 2, *Alternaxia*. 3 and 4, *Aspergillus*. 5, *Fusarium*. 6, 7, 8, *Mucor*. 9, 9a, *Penicillium*.

It is the *T. megalosporum endothrix* of *Sabouraud.*

The short, diseased fragmented hairs are mouldy looking. The spores are 5 to 6 microns.

T. sabouraudi.—Has a heaped-up festooned sort of culture. There is a similar fungus with a violet culture. These cause some of the scalp and beard ringworms.

It is easily dissociated in a 2 or 3% solution of caustic potash while *T. tonsurans* is hard to break up. The hairs are broken off close to the skin, hence "black dotted ringworm."

T. mentagrophytes.—This is the *T. megalsporon endoectothrix* of Sabouraud. The external spores are in chains or in short mycelial threads, not mosaics of spores, and are of very unequal size (3 to 15 microns).

The internal spores are scarce and are from 5 to 6 microns in diameter. To examine pull out downy hairs from the periphery of the lesion rather than the dead central ones. There are varieties from horse, cat, and bird. The lesions are more inflammatory than those of the endothrix class. Most of the beard and body ringworms belong to this group—very few scalp cases. The lesions are often of a pustular type. The cultures are finely rayed.

Some give yellow cultures, others white and one derived from birds a rose-colored culture.

Microsporum Audouini.—This is the so-called small-spored ringworm and is a very common and highly contagious affection of the scalp in children in England and France; less so in other countries.

It is almost never seen in the tropics. It almost exclusively affects the hairy scalp. The spores are 2 to 3μ in diameter. The broken stump of the hair is characteristic. The fungus is packed as a mosaic of spores, forming a white sheath, chiefly on the outside of the hairs. It gives a downy-white culture.

Achorion schoenleini is the cause of favus. The cultures are rather wrinkled. It is characterized by the scutulum or favus cup. This is a sulphur-yellow pea-sized cup with a central lusterless hair. Affected hairs may not show a cup. Favus is not so contagious as ringworm. It chiefly affects the hairy scalp, but may also invade the nails and even the body.

Microscopical examination shows great irregularity of spores and mycelium, the latter being irregularly disposed and of varying thickness and length and wavy instead of straight as in *Trichophyton.* There is also the greatest irregularity in the refractile favus spores—they are gnarled and bizarre shaped, in contrast to the regular ovals or spheres of the ringworm fungus. Cultures show ridges or convolutions.

Endodermophyton concentricum.—Castellani considers this as the causative fungus of tinea imbricata rather than *Aspergillus concentricus.*

It was formerly supposed that the causative fungus was *Aspergillus concentricus* but Castellani has demonstrated that fungi of this genus, when present, are merely accidental. He has isolated in cultures what he considers the causative fungus, *Endodermophyton concentricum.* He treated scales for ten minutes with absolute alcohol and then placed single scales in a series of tubes of maltose bouillon. The fungus grows between the rete malpighii and the external epidermal layers forming a network of mycelial threads, about 3 microns broad.

Another fungus cultured from tinea imbricata scales is *Endodermophyton indicum.* Inoculation of this organism in pure culture produced the disease.

The characteristics of the genus *Endodermophyton* are the growth of a mycelial

network between the rete malpighii and the superficial epidermal layers. In cultures only mycelial filaments are found, there are no conidia bearing hyphæ.

The fungus is also called *Trichophyton concentricum.*

Epidermophyton cruris. See *Microsporoides minutissimus*, page 160.

Under the name "dhobie itch" this fungus affection is probably better known to Europeans than any other tropical skin disease.

This name dhobie or washerman's itch has been given on account of associating it with the infection of the underclothing while being washed in the pools or streams along with the garments of those who have this skin disease. This, like every other widespread view, has probably some foundation but cannot be verified. It is the eczema marginatum of Hebra.

This affection is caused by various species of *Epidermophyton.* This genus differs from *Trichophyton* in that it never invades the hair or hair follicles.

The species which have been frequently reported are *Epidermophyton cruris*, *E. perneti* and *E. rubrum.* The mycelium is about 4 microns broad and the spores about 5 or 6 microns. All of these fungi can be cultured on Sabouraud's maltose agar, growth appearing in about a week, except *E. perneti*, which grows more rapidly.

IN THE SUBORDER CARPOASCEES we have to consider the family Perisporiacidæ. In this family the asci are completely inclosed by the investing membrane, the perithecium. When this rots the spores are set free. There are three genera of interest, *Penicillium*, *Aspergillus* and *Sterigmatocystis.*

In *Penicillium* we have vertical branches with strings of conidia. In Aspergillus these conidia arise from a globular termination of the hypha.

Penicillium.—While *Penicillium* does at times form perithecia, yet they characteristically show chains of spores. The common *P. glaucum* resembles a hand with terminal beads, more than the hair pencil, from which the name is derived.

P. crustaceum.—Is the common blue-green mould. It has been deemed pathogenic in cases of chronic catarrh of the Eustachian tube and in gastric hyperacidity.

P. montoyai.—Cause of violet pinta.

Aspergillus.—These have sterigmata carrying chains of spores, these sterigmata being little processes projecting out from the knob-like termination of the aërial hypha (columella). Of the pathogenic Aspergilli we have:

1. *A. fumigatus.*—This has been considered as the cause of pellagra. A pulmonary mycosis resembling phthisis may be due to this species.
2. *A. repens.*—This has been found in the auditory canal and may produce a false membrane.
3. *A. flavus.*—This has been found in the discharges of chronic ear diseases.
4. *A. concentricus.*—Formerly considered as the cause of an important tropical ringworm, tinea imbricata. The scales are dry, like pieces of tissue-paper.

There are generally about four rings which do not heal in the center. General appearance is that of watered silk. There are no inflammatory lesions. Common in Malay peninsula. Also found in some parts of the Philippines and in China. Some authorities consider the fungus to be a *Trichophyton*.

Castellani claims that the cause is a fungus which develops between the stratum corneum and the deeper layers of the epidermis, *Endodermophyton concentricum*. It differs from the achorions in not invading hair follicles. See page 156.

5. *A. pictor*.—This is the cause of a skin affection of Central America (Pinta). In the affection colored spots appear on the skin, chiefly on face, forearms, and chest. The disease is attended with a mangy odor. Spots are of various colors; if the superficial epithelium is affected we have a dark violet color. Deeper involvement gives red spots.

Other names for the disease are caraate and mal de los pintos. At first it was thought that the different colors shown by the eruption were due to varying depths of the proliferating fungi in the skin layers but it is now known that the explanation is in a variety of species in the different types of pinta.

The pure violet pinta is caused by *Aspergillus pictor*, while the grayish-violet one is due to *Penicillium montoyai*. A species of *Monilia* causes the white variety and different species of *Montoyella* a black and a red variety respectively. The genus *Montoyella* is stated by Castellani to have both slender and thick mycelial threads, from the thicker of which spring delicate hyphæ terminating in pear-shaped conidia.

Material scraped from the lesions and mounted in liquor potassæ shows the fructification terminations characteristic of *Aspergillus* or *Penicillium* in the violet or gray-violet varieties while the white, black and red ones only show mycelial threads and scattered spores. These pinta species of fungi can be cultivated on Sabouraud's medium.

Montoya thinks that the pinta fungi lead a saprophytic existence in the waters of mines or other places with a constant high temperature, and states that he has obtained pure cultures from such sources.

Sterigmatocystis.—This genus has chains of conidia, similar to those of *Penicillium*, but these are borne on other short chains, which arise from the clubbed aërial hyphæ (conidiophores). These are called secondary and primary sterigmata, respectively.

S. nidulans.—This fungus has been found in cases of otomycosis as well as in the white granules of mycetoma.

Hyphomycetes.—In this order are grouped certain genera which cannot properly be assigned to any of the other orders. They are also designated Fungi Imperfecti, for the reason that the fruiting bodies characteristic of the other orders have not been satisfactorily observed.

Discomyces bovis.—This is the well-known ray fungus, the cause of actinomycosis. In man it is at times found in chronic suppurative conditions attended with much granulation tissue. Such pus may show small yellow-gray granules about

the size of a pin's head. When spread out between two slides the central portion shows a network of mycelium with bulbous thread-like rays going to the periphery. The "clubs" at the periphery are degenerate structures and do not stain by Gram. The central mycelium is Gram-positive. This mould is essentially an anaerobe and should be cultivated in a deep glucose agar stab. It may also be cultivated in bouillon. In this it grows at bottom. Growth is dry and chalky. In diagnosis look for the little granules. Curetting of the sinuses may give the "ray fungus" when they are not found free in the pus.

Discomyces maduræ.—This is a ray fungus found in the yellow "fish-roe" granules of madura foot. The disease is caused by the penetration of certain species of fungi into the tissues of the foot, although rarely the hand or some other part of

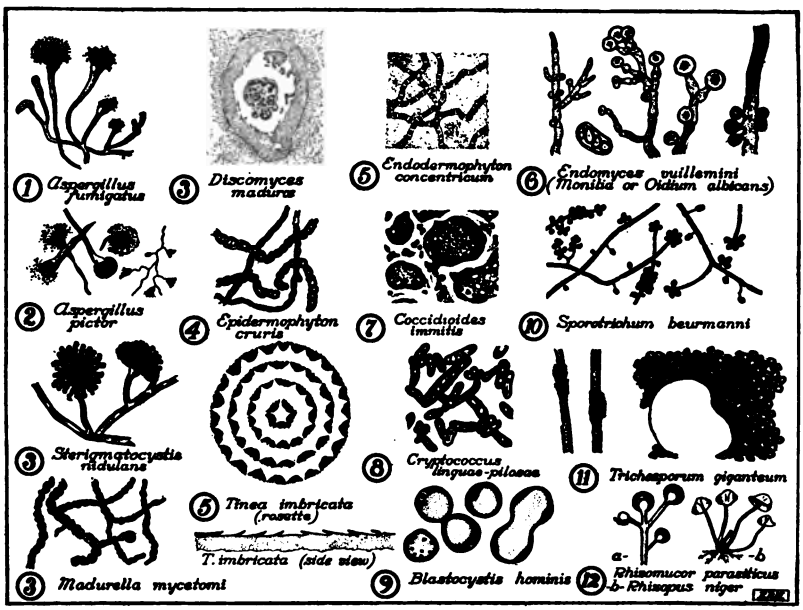


FIG. 43.—Important tropical fungi.

the body may be affected. These species of fungus develop in granulomatous areas from which sinuses lead to the surface of the foot, in the discharges from which are found small granules resembling those found in the discharges from actinomycosis lesions.

As a rule only one kind of fungus is found in a single case. The most common infection is that due to *Discomyces maduræ* (*Nocardia maduræ*) which is the fungus of the fish-roe like granules of the pale or white variety of mycetoma. These like the fungus of actinomycosis, *Discomyces bovis*, show a felted mycelium in the center and peripheral club-like structures. The granules are yellowish white and vary in size from a pin's head to a small pea. The mycelial threads are very narrow, 1 to

1½ microns. It grows aerobically and the cultures show slender mycelial threads which are Gram-positive. This is the organism of Carter's white mycetoma.

Other species of the pale, white or ochroid group of mycetoma fungi are *Indiella mansoni* (Brumpt's white mycetoma) *Nocardia asteroides* (Musgrave and Clegg's white mycetoma) *Sterigmatocystis nidulans* (Nicolle's white mycetoma) and several others.

The cases caused by the black varieties are more rare and are characterized by the presence in the discharge from the sinuses of black gunpowder-like grains.

These hard, brittle, irregular grains are caused by various species of fungi of which the best known is Carter's black mycetoma (*Madurella mycetomi*). This species was cultured by Wright and first shows a grayish growth, later becoming black. Other black varieties of mycetoma are due to various other fungi. Bouffard's black variety is caused by *Aspergillus bouffardi*. DeBeurmann's black mycetoma has as cause *Sporotrichum beurmanni*.

Besides the white and black varieties we also have a red variety of mycetoma. The fungus grains are quite small and reddish in color. It is not an uncommon infection in certain parts of Africa, as Senegal. The cause is *Nocardia pelletieri*. *Discomyces carougeani* has been reported as the cause of juxta-articular nodes, but Breinl has been unable to verify the finding.

Malassezia furfur.—This is the fungus of tinea versicolor. It is common both in temperate and in tropical climates. It is characterized by dirty yellow spots about covered parts of the body. Scrapings show a profusion of mycelial threads and interspersed spores. It is very difficult to cultivate. The organism usually termed the bottle bacillus is really a fungus having the characteristics of the genus *Malassezia*. It is thought to be the cause of pityriasis of the scalp.

Microsporoides minutissimus.—This is generally considered as the cause of Erythrasma or dhobie itch, a very common intertrigo of the tropics. It is characterized by its narrow mycelium and small spores. Various fungi are found in this affection. Castellani considers the chief cause of dhobie itch to be a trichophyton. *Epidermophyton cruris*.

Clinically this affection shows festooned areas of a bright red color which tend to clear up in the center becoming fawn color. As a result of the intolerable itching and scratching the affection tends to spread from its favorite sites—the inner surfaces of the thighs and the scrotum. The spores and mycelium are abundant at the onset but later, one may not find any evidence of the mould. In some of the rapidly spreading cases I have found a symbiosis of fungus and coccus, the bacterial elements lying packed in aggregations scattered through the mycelial ground work.

Culturally these cocci were *S. pyogenes aureus*. See page 157.

Monilia albicans (*Oidium albicans*).—Castellani separates *Monilia* from *Endomyces* in that it does not show the asci and internal spores of the latter. In cultures it gives budding yeast-like growths and mycelial threads. On Sabouraud's medium it gives a whitish growth. It slowly liquefies gelatin and blood-serum and, after acidifying, clots milk. It is recognized as the organism of thrush. Bahr found this

fungus in the deep layers of the tongue as well as in the œsophageal and intestinal coatings of sprue.

Ashford is convinced that a species of *Monilia* which he is sure is distinct from *M. albicans,* is the cause of sprue. He states that this fungus is common in the bread of Porto Rico. He has recovered the organism from sprue lesions and has produced a monilia septicæmia in rabbits inoculated with the sprue fungus. This fungus is quite pathogenic when first isolated from sprue lesions.

In internal organs the mycotic areas are not associated with pus formation. On two occasions he has produced stomatitis in animals by feeding experiments.

Monilia tropicalis.—Castellani has reported this fungus as the cause of a broncho-mycosis. It does not coagulate milk nor liquefy gelatin. The growth on Sabouraud's medium is mainly of yeast-like cells.

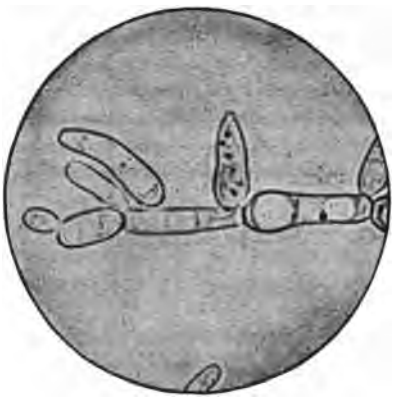


FIG. 44.—Thrush fungus. (*Kolle and Wassermann.*)

M. candida.—This fungus was found in white patches on tongue of a child. The conidia are 7 to 8 microns and the mycelium 1 to 1½ microns in diameter.

Boggs has recently isolated a *Monilia* which he considers as closely related to *M. candida.* The patient was at first thought to have a mammary carcinoma with axillary gland metastases. Later on there was a severe cough with abundant reddish-gray sputum which showed mycelium and yeast-like cells. Cultures on glucose agar, potato, etc., at 37°C. and at room temperature gave a moist glistening whitish growth which, when examined, showed only yeast-like cells, no mycelial growth. Hyphæ only showed in later cultures.

There was a fair growth in milk with after three or four weeks an alkaline reaction and firm coagulum. Slight acid production in glucose but none in lactose, saccharose or mannite.

Mycelial growth was more rapid in anaerobic cultures than in aerobic ones. Boggs notes that his *Monilia* is morphologically indistinguishable from the *Monilia* of sprue. The buccal mucosa of this case did not show any abnormalities.

Trichosporum giganteum.—This is the cause of a disease of the hairs, known in Columbia as "Piedra," so called from the small gritty-like masses along the length of the hair. These spores are arranged like mosaics about the hair.

Sporotrichum beurmanni.—This fungus has a narrow mycelium (2μ) and branches in all directions. The spores appear as little grape-like clusters of oval spores (3 to 5μ) at the end of a filament. It is readily cultivated, showing as a small white growth about the eighth day.

The fungus of sporotrichosis develops in tissue by budding, not showing the mycelial growth seen in artificial cultures. Potato makes a good medium and often such cultures show pigmentation.

This mould produces indolent, glistening, subcutaneous tumors which are painless. They may ulcerate and give off a brownish discharge. They resemble tuberculous or syphilitic lesions.

Certain organisms which resemble both moulds and bacteria, having branching filamentous forms and at the same time having a spore-like method of reproduction, are known under the names *Streptothrix* or better *Nocardia.* It is chiefly in various pathological processes of the lungs that they have been observed, but in addition they have been noted in brain, glands, kidney and subcutaneous tissue.

The infections are most likely to be confused with phthisis and glanders. The organisms are easily cultivated and in staining reactions are midway between T. B. and Actinomycosis.

Castellani uses the generic name *Nocardia* for *Discomyces.*

Diagnosis of Fungi

The most expeditious way to examine for fungi is to treat the scales or hairs with a 10% solution of caustic potash or soda. Then crush between two slides; heat moderately over the flame and examine.

Tribondeau's method is to treat the scales with ether, then with alcohol, and finally with water. Next put the sediment (it is convenient to use a centrifuge) in a drop of caustic soda solution. Cover with a cover-glass, and after the preparation has stood about an hour run glycerine under the cover-glass.

A very satisfactory method is to scrape the scales with a small scalpel, and smear out the material so obtained in a loopful of white of egg or blood-serum on a glass slide. By scraping vigorously the serum may be obtained from the patient. After the smear has dried, treat it with alcohol and ether to get rid of the fat. It may then be stained with Wright's stain or by Gram's method. The ordinary Gram method may be used or the decolorizing may be done with aniline oil, observing the decolorization under the low power of the microscope.

Yeasts are best examined in hanging drop on the plain slide with vaselined cell, as given under "Blood."

An excellent way to examine moulds is to seize some of the projecting sporangia from the surface of a plate with forceps and mount in liquid petrolatum. I have found that moulds in scales from skin or infecting various mites or insects will show a growth in this medium when mounted on a slide and covered with a cover-glass.

Cultivation of Fungi

Moulds grow well on media with an acid reaction, so that by adjusting the reaction to +2% or even higher, we permit of the growth of the fungi, but inhibit bacterial development.

Glycerine agar, bread paste, or potato media are all suitable, but the best medium is that of Sabouraud:

Maltose	4.0 grams.
Peptone	1.0 gram.
Agar	1.5 grams.
Water	100.0 c.c.

Make the reaction about +2.

Before inoculating media with moulds, some recommend placing the material in 60% alcohol for one or two hours to kill the bacteria. The moulds withstand such treatment.

In cultivating moulds it is best to use small Erlenmeyer flasks, containing about ¼ in. of media on the bottom, for the development of the colonies. In order to separate the mould we may take the hair or scales on a sterile slide and cut them into small fragments with a sterile knife. Then moisten a platinum loop from the surface of an agar slant, touch a fragment with the loop, and when it adheres transfer it to the agar slant. Make four or five inoculations on the surface and from suitable growth, after four to seven days, inoculate the medium in the Erlenmeyer flask. Esmarch roll cultures are better than flask ones.

Plauth recommends receiving the mould material be ween two sterile glass slides. Seal the edge of the lide with wax and place the preparation in a moist chamber for four to seven days. From developing fungus growth inoculate the medium in the Erlenmeyer flask. A Petri dish containing several layers of thoroughly moistened filter-paper in top and bottom makes a satisfactory moist chamber.

For the study of the morphology of *Monilia* in cultures, Boggs used stab cultures of 15% gelatin. The growth in the tube was hardened in 10% formalin, the glass cracked off and sections of the gelatin column cut across at any desired level. These blocks were sectioned with the freezing microtome; stained in dilute aqueous fuchsin (1 to 30) for several hours; then differentiated in saturated solution of citric acid until nearly decolorized. The sections were floated on slides, air dried without blotting, cleared in xylol and mounted in balsam.

For staining fungi in sections of tissue Busse recommends the following method: 1. Hæmatoxylin, 10 to 15 minutes then wash in tap water. 2. Carbol fuchsin (1 to 20) 30 minutes or over night. Decolorize in alcohol for a few minutes then through absolute alcohol and xylol to mount in balsam. The moulds are red.

CHAPTER XI

BACTERIOLOGY OF WATER, AIR, MILK, ETC.

Bacteriological Examination of Water

While in a chemical examination as to the character of a water there are certain relations between the free and albuminoid ammonias, nitrates, nitrites, chlorides, etc., which indicate the probable animal as against vegetable nature of the organic matter present, yet it is a more or less presumptive evidence. In a bacteriological examination of water the finding of the colon bacillus may from a practical standpoint be considered as positive evidence of human fæcal contamination. Theoretically, the possibility of organisms being present corresponding culturally to *B. coli* and derived from cereals is to be considered. Also the fæces of animals contain an organism which cannot be differentiated from the colon bacillus.

In detectin sewage contamination in water to which varying amounts of sewage had been added, it was found that the bacterial tests were from 10 to 100 times more delicate than the chemical ones.

As showing sewage contamination of water, the presence of the *B. coli* has been generally accepted as the most satisfactory indication. The English authorities consider sewage streptococci and the spore-bearing *B. enteritidis sporogenes* as of value as indicators as well as the *B. coli*—the presence of sewage streptococci indicating very recent sewage contamination and that of the *B. enteritidis sporogenes*, in the absence of streptococci and colon bacilli, as evidence of sewage contamination at some period more or less remote.

In the United States the colon bacillus alone is considered the indicator of sewage contamination, and all tests, presumptive or positive, are based on the presence of this organism.

It is not the finding of the colon bacillus but rather the question of its relative abundance that is involved in a water analysis. Thus the finding of one colon bacillus in 50 c.c. of water would not have weight as showing contamination, but the presence rather constantly of the colon bacillus in 1 c.c. or less makes contamination of a water supply probable.

In collecting samples of water for bacteriological examination, the following points should be considered:

1. The bottles, which should have a capacity of from 25 to 100 c.c., should be sterile. Sterilization may be effected by heat or by rinsing with a little sulphuric acid and subsequently washing out thoroughly with the suspected water before collection. The utmost care must be exercised that the fingers do not come in contact with the glass stopper of the neck of the bottle while filling it. If the specimen is to be sent some distance, it should be packed in ice to prevent bacterial development. Frank and states that a count of 1000 became 6000 in six hours and 48,000 in forty-eight hours. In water packed in ice for a considerable time, however, the bacterial count may diminish.

2. If collecting from city water supplies, secure the sample direct from the mains and let the water run from the tap a few minutes before collection. If the water be taken from a pond, stream, or cistern, be sure that the specimen comes from at least 10 inches below the surface. As sedimentation is the most important method in self-purification of rivers and ponds, it will be understood that any stirring up of the mud on the bottom will enormously increase a bacterial count.

Quantitative Bacteriological Examination

1. Deliver definite quantities of the water to be examined into tubes of liquefied gelatin or agar and plate out the same in a series of Petri dishes.

A more practical method is to deliver the water from the graduated pipette into the empty sterile dish. The water should be deposited in the center of the plate and the melted gelatin or agar poured directly on the water and then, carefully tilting to and fro, mix the water and the media. One set of plates should be of gelatin and incubated at room temperature; a similar set should be of lactose litmus agar and incubated at 38°C. If the water is highly contaminated, it is necessary to dilute it; thus, with river water, which may contain from 2000 to 10,000 bacteria per c.c., a dilution of 1 to 100 would be desirable.

Ordinarily it will be sufficient to deliver from a sterile graduated pipette 0.2, 0.3, and 0.5 c.c. of the water in each of two sets of plates: one set for gelatin, the other for agar.

When gelatin is not at hand or convenient to work with, the gelatin plates may be replaced by others of lactose litmus agar for incubation at room temperature. After twenty-four hours at 38°C. or forty-eight hours at 20°C., the count should be made.

Example.—Forty colonies were counted on the gelatin plate containing 0.2 c.c. ($\frac{1}{5}$) of the water. The number of organisms would be 200 per c.c. Ten colonies were counted on the agar plate containing 0.2 c.c. and incubated at 38°C. Number of bacteria developing at body temperature equals 50 per c.c.

There is no strict standard as to the number of bacteria a water should contain per c.c. Koch's standard of 100 colonies per c.c. is generally given. It is by the qualitative rather than the quantitative analysis that one should judge a water.

If there should be very many colonies on a plate, the surface can be marked off into segments with a blue pencil. If very numerous, cut out of a piece of paper a space equal to 1 sq. cm. By counting the number of colonies inclosed in this space

at different parts of the plate, we can strike an average for each space of 1 sq. cm. To find the number of such spaces contained in the plate, multiply the square of the radius of the plate by 3.1416. Then multiply this number by the average per square centimeter, and we have the total number of colonies on the plate. This is the principle of the Jeffers disc.

The relative proportion between the bacterial count at 20°C. and that at 38°C. is of great importance from a qualitative standpoint, as will be seen later.

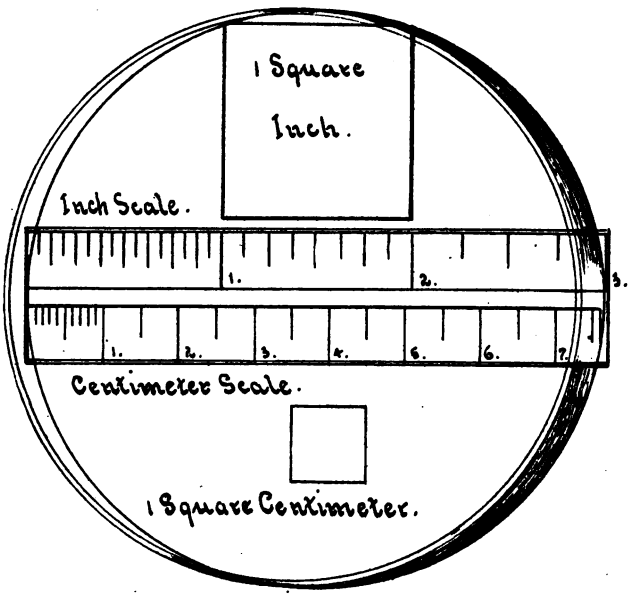


FIG. 45.—Estimating colonies on water plate. The diameter of the bottom of this Petri dish is 3 inches or 7.5 + centimeters. The area of a circle is equal to the square of the radius multiplied by π or $3\frac{1}{7}$. $1\frac{1}{2}$ in. =radius, $1\frac{1}{2} \times 1\frac{1}{2} = 2.25$. $2.25 \times 3\frac{1}{7} = 7.07$ square inches. 3.75 cm. =radius. $3.75 \times 3.75 = 14.06$. $14.06 \times 3\frac{1}{7} = 44.1$ square centimeters. Number of bacterial colonies in 1 sq. in. averages, approximately, 75. Number in 7.07 sq. in. = 530. Number of bacterial colonies in 1 sq. cm. averages, approximately, 12. Number in 44.1 sq. cm. = 528.

2. Deliver into a series of Durham fermentation tubes containing glucose bouillon and into another series containing lactose bouillon varying definite amounts of the water to be examined. In tubes showing the presence of gas in both glucose and lactose bouillon the evidence is presumptive that the colon bacillus is present. For the positive demonstration plates must be made from such tubes as show gas.

It is sufficient to deliver from graduated pipettes in each series quantities of water varying in amount from 0.1 c.c. to 10 c.c. In our laboratory we inoculate with 0.1 c.c., 0.2 c.c., 0.5 c.c., 1 c.c. and 10 c.c. of the suspected water. If the 0.1-c.c. tubes show gas, we have reason to assume that the water contained at least 10 colon

bacilli per c.c. If only 10 c.c. tubes showed gas—those with less amounts not having gas—we would be in a position to state that the water contained the colon bacillus in quantities of 10 c.c., but not in quantities of 1 c.c. or less. Many authorities regard water as suspicious only when the colon bacillus is present in quantities of 10 c.c. or less; waters of good quality frequently showing the presence of the colon bacillus in quantities of 100 to 500 c.c.

It is generally accepted that if a water shows the presence of the colon bacillus in quantities of 1 c.c. or less, it should be regarded as suspicious.

At the present time the medium that gives the least source of error in carrying out the quantitative presumptive tests is the lactose bile. It is made by adding 1% of the lactose and 1% of peptone to ox bile, and fermentation tubes of the media showing gas may be considered as very probably containing the colon bacillus. The percentage of error with this method is reported to be only 11%, while with glucose fermentation tubes the error is more than 50%. Gas formation is usually shown in forty-eight hours, but it is advisable to continue the incubation for seventy-two hours. These presumptive tests are chiefly of value in highly contaminated waters. Even with this method plates should be made.

3. As the colon and sewage streptococci ferment lactose with the production of acid and hence produce pink colonies on lactose litmus agar, much information can be obtained from the proportion existing between the number of pink colonies and those not having such a color. Waters of fair degree of purity rarely give any pink colonies.

Qualitative Bacteriological Examination

General Considerations.—In some countries the proportion of liquefying to nonliquefying colonies on gelatin plates is considered of importance. Certain sewage organisms belonging to the proteus and cloaca groups liquefy gelatin; consequently, if the proportion of liquefying to nonliquefying be greater than as 1 to 10, the water is considered suspicious. The test is not considered by American authorities as of any particular value.

The American Public Health Association recognizes the importance of the information obtained from a comparison of the number of organisms developing at 38°C. and those developing at 20°C. Bacteria whose normal habitat is the intestinal canal naturally develop well at body temperature, while normal water bacteria prefer the average temperature of the water in rivers and lakes. Consequently when the number of organisms developing at 38°C. at all approximates the number developing at 20°C., there is a strong suspicion that sewage organisms may be present. Normal waters give proportions of 1 to 25 or 1 to 50, while in sewage contaminated waters the proportion may be as 1 to 4 or 1 to 2.

In addition, the appearance of pink colonies on the lactose litmus agar is of great assistance in judging of a water. Both sewage streptococci and the colon bacillus give pink colonies—those of the streptococci are smaller and more vermilion in color. Microscopic examination will differentiate the cocci from the bacilli. It is well to bear in mind that the pink colonies after twenty-four hours may turn blue in forty-eight hours from the development of ammonia and amines. Consequently the lactose litmus agar plates should be studied after twenty-four hours.

A good water supply will rarely show a pink colony, while in a sewage-contaminated one the pink colonies will probably predominate.

A commission composed of eminent American bacteriologists and sanitarians have recommended the following as maximum limits of bacteriological impurity:

1. The total number of bacteria developing in standard agar plates, incubated twenty-four hours at 37°C.; shall not exceed 100 per c.c. Provided that the estimate shall be made from not less than two plates, showing such numbers and distribution of colonies as to indicate that the estimate is reliable and accurate.

2. Not more than one out of five 10-c.c. portions of any sample examined shall show the presence of organisms of the *Bacillus coli* group when tested as follows:

(*a*) Five 10-c.c. portions of each sample tested shall be planted, each in a fermentation tube containing not less than 30 c.c. of lactose peptone broth. These shall be incubated forty-eight hours at 37°C. and observed to note gas formation.

(*b*) From each tube showing gas, more than 5% of the closed arm of fermentation tube, plates shall be made, after forty-eight hours' incubation, upon lactose litmus agar or Endo's medium.

(*c*) When plate colonies resembling *B. coli* develop upon either of these plate media within twenty-four hours, a well-isolated characteristic colony shall be fished and transplanted into a lactose-broth fermentation tube, which shall be incubated at 37°C. for forty-eight hours.

For the purpose of enforcing any regulations which may be based upon these recommendations the following may be considered sufficient evidence of the presence of organisms of the *Bacillus coli* group.

First.—Formation of gas in fermentation tube containing original sample of water.

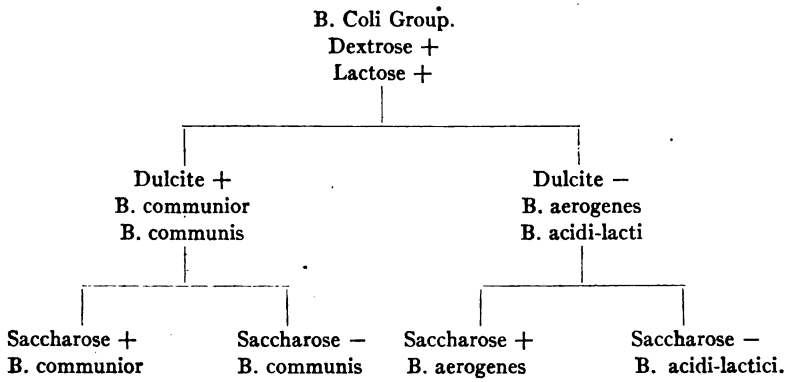
Second.—Development of acid-forming colonies on lactose litmus agar plates or bright red colonies on Endo's medium plates, when plates are prepared as directed above under (*b*).

Third.—The formation of gas, occupying 10% or more of closed arm of the fermentation tube, in lactose-peptone-broth fermentation tube, inoculated with colony fished from twenty-four-hour lactose litmus agar or Endo's medium plate.

These steps are selected with reference to demonstrating the presence in the sample examined of aerobic lactose-fermentating organisms.

3. It is recommended, as a routine procedure, that in addition to five 10-c.c. portions, one 1-c.c. portion, and one 0.1-c.c. portion of each sample examined be planted in a lactose-peptone-broth fermentation tube, in order to demonstrate more fully the extent of pollution in grossly polluted samples.

The members of the B. coli group are separated by the American Public Health Association according to fermentation activities as follows:



Further divisions are made by the use of mannite and raffinose, giving varieties of the above four species.

Dulcite, like mannite, is a hexatomic alcohol. It is isomeric with mannite and is present in Madagascar manna.

Raffinose is a trisaccharide; maltose, lactose and saccharose are disaccharides, while dextrose and lævulose (hexoses) arabinose and xylose (pentoses) are monosaccharides.

The diagnostic characteristics considered important by the American authorities in reporting the colon bacillus (recently designated excretal colon bacillus) are:

1. Typical morphology, nonsporing bacillus, relatively small and often quite thick.

2. Motility in young broth cultures. (This is at times unsatisfactory, as some strains of the colon bacillus do not show it even in young bouillon cultures.)

3. Gas formula in dextrose broth. Of about 50% of gas produced, one-third should be absorbed by a 2% solution of sodium hydrate (CO_2). The remaining gas is hydrogen. (Later views indicate that the gas formula is exceedingly variable and should not be depended upon. To carry out this test one fills the bulb of a fermentation tube with the caustic soda solution, holding the thumb over the opening or with a rubber stopper, the bouillon culture and the soda solution are mixed by tilting the fermentation tube to and fro. The total amount of gas is first recorded and then that remaining after the CO_2 has been absorbed is reported as hydrogen.)

4. Nonliquefaction of gelatin.

5. Fermentation of lactose with gas production.

6. Indol production.

7. Reduction of nitrates to nitrites.

To these may be added the acidifying and coagulation of litmus milk without subsequent digestion of the casein. The production of gas and fluorescence in glucose neutral red bouillon is also a very constant function of the colon bacillus. *B. coli aerogenes* is similar to *B. coli* with the exception of nonmotility, formation of gas in starch media (bubbles on potato slant) and frequent lack of indol production. It is often, especially in milk cultures, provided with a capsule and rarely forms chains. It is a member of the Friedländer group but differs from the typical pneumobacillus by producing acid and gas in lactose broth and by its coagulation of milk.

B. coli anaerogenes is also similar to *B. coli* but does not produce gas in glucose and lactose. This latter organism is not usually recognized by American authorities but I have found on Endo plates an organism showing the red colony with metallic luster which failed to produce gas in either glucose or lactose.

NOTE.—The reduction of neutral red with a greenish-yellow fluorescence is very striking and has been suggested as a test for the colon bacillus. Many other organisms, especially those of the hog cholera group, have this power. It is convenient, however, to color glucose bouillon with about 1% of a ½% solution of neutral red.

On the plates made for the detection of colon bacillus may be found certain organisms which have origin in fecal contamination. The more important of these are those of the paratyphoid, cloaca and proteus groups. In addition, the *B. fecalis alkaligines* has not rarely been isolated. Among natural water bacteria there may be present either the liquefying or the nonliquefying *B. fluorescens*. These colonies have a yellowish-green fluorescence.

Certain chromogenic cocci and bacilli are found in uncontaminated waters as *B. indicus* or *B. violaceus*. From surface washings we obtain certain soil bacteria as *B. mycoides*, *B. subtilis*, *B. megatherium*. One of the higher bacteria which shows long threads, *Cladothrix dichotoma*, is common, and is characterized by a brown halo around its gelatin plate colony.

Isolation of the Typhoid Bacillus from Water

This is probably the most discouraging procedure which can be taken up in a laboratory. Only the most recent reports of such isolation from water supplies, which have been verified by immunity reactions, can be accepted and of these the number of instances is exceedingly small. Owing to the long period of incubation, the typhoid organisms may have died out before the outbreak of an epidemic suggests the examination of the water supply.

There have been various methods proposed for the detection of the *B. typhosus* in water. A method which would offer about as reasonable a chance of success as any other would be to pass 2 or 3 liters of the water through a Berkefeld filter; then to take up in a small quantity of water all the bacteria held back by the filter. Then plate out on lactose litmus agar and examine colonies which do not show any pink coloration. The dysentery bacillus has about the same cultural characteristics as the typhoid one, so that it is important to note motility. If from such a colony you obtain an organism giving the cultural characteristics of *B. typhosus*, carry out agglutination and preferably bacteriolytic tests as well. Some strains of typhoid, especially when recently isolated from the body, do not show agglutination.

The Conradi Drigalski, the malachite-green, and various caffeine containing plating media have been highly recommended.

Isolation of the Cholera Spirillum from Water

The method proposed by Koch in 1893 does not seem to have been improved upon by later investigators. To 100 c.c. of the suspected water add 1% of peptone and 1% of salt. Incubate at 38°C., and at intervals of eight, twelve, and eighteen hours examine microscopically loopfuls taken from the surface of the liquid in the flask. So soon as comma-shape organisms are observed, plate out on agar. The colonies showing morphologically characteristic organisms should be tested as to agglutination and bacteriolysis. Inasmuch as the true cholera spirillum shows a marked cholera-red reaction it is well to inoculate a tube of peptone solution from such a colony and add a drop of concentrated sulphuric acid after incubating for eighteen hours. The rose-pink coloration is given by the cholera spirillum with the acid alone—the nitroso factor in the reaction being produced by the organism.

Bacteriological Examination of Milk

A bacterial milk count is of comparatively little value as showing whether a milk is dangerous or not. As a matter of fact, a milk which contains several million of bacteria per c.c. might be less dangerous than one containing only a few thousand, especially if in the latter there were numerous liquefiers and gas producers present. There is, however, one point of importance in connection with the quantitative

estimation of bacteria in milk, and that is the fact that in order to keep the development of the bacteria within the limits of 10,000 to 50,000 per c.c., it is necessary that the requirements of cleanliness in milking and the rapid cooling of the milk after obtaining it and the keeping of the temperature below 50°C. be rigidly observed. If a milk has a high count it shows some error in the handling of the milk. Anderson has found that top milk contains from 10 to 500 times as many bacteria as bottom milk. Centrifugally raised cream contains more bacteria than that forming by gravity. In making a quantitative bacteriological examination, the principle is the same as with water.

Make a known dilution of the milk with sterile water; add definite quantities of this diluted milk to tubes of melted agar or gelatin, and pour into plates. The diluted milk may also be delivered in the center of the plate and the melted agar or gelatin poured directly on it, mixing thoroughly. Always shake the bottle well before taking sample.

Example.—Added 1 c.c. of milk to 199 c.c. of sterile water in a large flask (500 to 1000 c.c.). After shaking thoroughly, take 1 c.c. of this 1:200 dilution and add it to 99 c.c. of sterile water. Shaking thoroughly, we have a dilution of 1: 20,000 Of this we added 0.5 c.c. to a tube of gelatin or agar. After incubation the plate showed 75 colonies. Therefore the milk contained in each c.c. $75 \times 2 \times 20{,}000$ (dilution) $= 3{,}000{,}000$—the number of bacteria in each c.c. of milk.

Lactose litmus gelatin or agar is to be preferred in milk-work, as the normal lactic acid bacteria produce reddish colonies which are very striking. A standard easily attained for high-grade, certified milk would be 5000 to 10,000 per c.c.

In the qualitative examination of milk, many dairies employ the fermentation tube, any organism producing gas being considered undesirable. Again liquefying organisms, as shown by the presence of such bacteria in the gelatin plates, are evidence of probable contamination by fæcal bacteria. A question which seems difficult to decide is as to the general nature of the so-called normal lactic acid bacteria of milk. Some describe them as very short, broad bacilli with very small colonies, fermenting lactose with the formation of lactic acid. Others consider that the streptococci are the organisms which are concerned with the normal fermentative changes. In examining specimens of milk considered the best on the market, I have repeatedly found the small red colonies on lactose litmus agar to be in chains of either Gram-positive streptococci or streptobacilli.

Acid Producing Organisms.—Shippen considers the chief organism concerned in the souring of milk as *B. güntherii*, but notes that it is the same organism as *S. lacticus*. All authors note the difficulty of deciding whether the morphology is coccal or bacillary. McGuire found these organisms almost constantly present in the dung of cows. The organisms he obtained from cow dung were chiefly members of the coli-aerogenes group and *S. lacticus*.

Of the acid-forming bacilli in milk we have 1. the *B. lactis acidi* group. These are oval cells about 0.9 microns by 0.6 microns, often in chains. They are Gram-positive and nonmotile. They may be the same as *Streptococcus lacticus* of Kruse. They curdle milk with a homogeneous clot—this being due to the fact that they do not produce gas in lactose media. 2. The *B. coli aerogenes* group. These are gas producers. (See under water.) 3. The *B. bulgaricus* group. In connection with the organisms present in the tablets used for treating milk to produce lactic acid for the treatment of intestinal disorders, and considered to be normal lactic acid bacteria, I have found both streptococci and bacilli. These have all agreed, however, in not producing gas in either lactose or glucose fermentation tubes.

I have often found the commercial fluid cultures sterile, the great acidity produced by *B. bulgaricus* causing this. Fresh tubes may show an acidity of +12 or about 10 times that of ordinary culture media.

The organism upon which special stress is laid in these so-called lactic acid producers is the *B. bulgaricus*. This is a large, nonmotile organism with square ends like anthrax. It often occurs in long chains and does not possess spores. It is Gram-positive and often shows metachromatic granules like those of the diphtheria bacillus. Colonies show in forty-eight hours which resemble streptococcus ones, but are more contoured on the surface. Magnified the colonies resemble young mould colonies. It grows better on milk agar plates than on whey agar plates. The opacity of the milk agar plate is but a slight objection. It produces a deep vivid pink in litmus milk, while milk streptococci only cause a light pink. It produces a very large amount of acid (3%). Little or no growth on ordinary laboratory media or below 20°C. (Op. temp. 42°C.).

Heinemann states that it occurs normally in human fæces and various fermented milks—also in gastric juice when HCl is absent. To isolate, put milk or fæces into a broth containing 0.5% acetic acid and 2% glucose. Transfer to litmus milk after twenty-four hours and from such tubes plate out on milk serum agar (coagulate boiling milk with a few drops of acetic acid, filter and add 1% peptone, 2% glucose and 1.5% agar).

As they grow in very acid media the term acidophilous is applied. It was supposed that these bacteria were peculiar to certain fermented milks as matzoon and yogurt. Hastings has shown the group to be present in milk in the United States and considers the source to be the alimentary tract of cows.

Milk Leukocytes.—Another source of information as to the quality of a milk may be derived from a study of the number of leukocytes or pus cells contained in 1 c.c. of the milk. It must be understood that cellular elements which differ only slightly from true pus cells may be found in the milk of healthy cows and may be found in great numbers. Statements have been made that such cells are neither amœboid nor phagocytic.

The Doane-Buckley method is probably the most accurate. In this you throw down the cellular contents of 10 c.c. of milk in a centrifuge revolving about 1000 times a minute for ten to twenty minutes. Then remove supernatant milk and add 0.5 c.c. of Toisson's solution to the sediment. Instead of Toisson's solution I use Gram's iodine solution which brings out the leukocytes equally well and gives a cleaner preparation. You thus have the leukocytes of 10 c.c. contained in 0.5 c.c. (Concentrated 20 times.) Make a hæmatocytometer preparation as for blood and find the average number of cells for each square millimeter. Then multiply this by 10 to get the number of cells in a cubic millimeter. As a cubic millimeter is 1000 times smaller than a cubic centimeter, you multiply the number per cubic millimeter by 1000. Then, as the milk was concentrated 20 times, you divide by 20. (If it were diluted 20 times, you would multiply by 20.)

Example.—Found an average of 50 cells per square millimeter. This would make 500 per cubic millimeter, and 500,000 per c.c.; then 500,000 divided by 20 would give 25,000.

There is no agreement as to a standard for allowable leukocytes. Even in apparently healthy animals they may exceed 100,000 per c.c. Doane has suggested 500,000 per c.c. as a preferable limit.

The smear methods for determining the number of leukocytes present do not compare in accuracy with the volumetric ones. It is important, however, from a standpoint of examining for tubercle bacilli, etc., as well as for recognition of leukocytes, to deposit the sediment from a centrifuge tube, taken up with a capillary bulb pipette, on a glass slide. Smear out slightly and then when dry fix with a mixture of ether and absolute alcohol. Flood with ether to get rid of remaining fat and stain by Gram's method or by acid-fast staining.

To summarize, we may state that the bacterial count is an indicator of the care used in handling the milk while the presence of harmful bacteria (qualitative examination) or numerous pus cells indicates disease in the cow. During 1912 severe epidemics of sore throat due to a streptococcus, *S. epidemicus*, were traced to milk of cows having probably suffered from mastitis. In Baltimore the milk had been pasteurized by the flash method which indicates the unreliability of this process.

It has now been determined that these streptococci were of human and not bovine origin; milkers with streptococcic infections contaminating the cow's udder. While experiments have shown that certain streptococci may resist the pasteurizing temperature (145°F. for thirty minutes) yet with pathogenic streptococci of human source such a temperature has been entirely effective. This would show that milk properly pasteurized is safe from a standpoint of causing streptococcal sore throat.

Pasteurization of Milk.—The objections to this method of preserving milk have been (1) that the lactic acid bacteria which have been by some credited with antagonism to harmful bacteria, would be destroyed by pasteurization, (2) the more

rapid development of bacteria in milk that has been pasteurized, (3) interference with nutritive qualities and (4) pasteurized milk does not show its deterioration as does unpasteurized milk, thus failing to give a clue as to the age of the milk.

The United States Bureau of Animal Industry in studying this important phase of the milk question has grouped the milk bacteria into three classes (a) acid-forming, (b) putrefactive (liquefying) and (c) inert bacteria. In their investigations it was found that many acid-forming bacteria withstood temperature as high as 168°F., so that pasteurized milk was soured just as is raw milk, but more slowly. They found that pasteurized milk showed fewer putrefactive bacteria than raw milk, so that even should it be a fact that injurious toxins were produced by spore-bearing putrefactive organisms the development of such organisms would be even less in pasteurized milk.

The statement so often advanced that bacteria develop more rapidly in pasteurized milk than in raw milk was proved fallacious.

It was recommended that holding the milk for thirty minutes at 145°F. was a far better method of pasteurizing than quickly bringing the milk to a temperature of 185°F. (flash method). All admit the great value of the killing of important pathogens (typhoid, cholera, streptococci, etc.).

Bacteriological Examination of Air

In Paris a cubic meter of air was found to contain the following number of organisms:

Suburbs.—Winter,	145 moulds,	170 bacteria.
Summer,	245 moulds,	345 bacteria.
City Hall.—Winter,	1345 moulds,	4305 bacteria.
Summer,	2500 moulds,	9845 bacteria.

Air of hospitals, especially after sweeping, may contain 50,000 bacteria per cubic meter. There does not seem to be any particular relation between the amount of carbon dioxide in air and the bacterial content.

Petri's Rough Method.—Exposure of a lactose litmus agar plate (capacity 100 sq. cm.) for five minutes will give the number of organisms present in 10 liters of air. Multiply by 100 for 1 cu. m.

The two groups of organisms usually found in air are 1. bacteria and 2. moulds. Moulds (spores) may be carried by currents of air; bacteria, however, are generally carried about by particles of dust or finely divided liquids (spray). On the lactose litmus agar plate staphylococci and streptococci show as bright red colonies.

Sedgwick-Tucker Sterile Granulated Sugar Method.—Sterilize aerobioscope and introduce granulated sugar on support. Again sterilize (not over 120°C. in dry-air sterilizer). Allow a given quantity of air to pass through; then shake the sugar into wide part of aerobioscope. Now pour in 10 or 15 c.c. of melted gelatin

(40°C.) to dissolve sugar. Roll tubes as for Esmarch roll cultures, and incubate at room temperature. To draw air through the aerobioscope, connect the small end with a piece of rubber tubing which is attached to a tube in the stopper of an aspirating bottle. Having poured a definite quantity of water into the aspirating bottle, allow the water to run out. The same quantity of air will be drawn through the sugar of the aerobioscope as the amount of water passing out of the aspirating bottle. The bacteria and moulds are caught by the sugar.

FIG. 46.—Sedgwick-Tucker aerobiscope. (*Mac Neal.*)

Example.—Passed 10 liters of air through the aerobioscope. The bacteria in this quantity of air showed 75 colonies when incubated at 20°C. The unit being 1 cu. m. or 1000 liters, we have only obtained the bacteria of one hundredth of the unit. Hence multiplying 75 by 100 gives 7500 bacteria as present in 1 cu. m. of the air examined.

A very satisfactory method is to take a test-tube containing 5 c.c. of sterile water and having a rubber stopper with two perforations, one for a long piece of glass tubing which dips down into the water and a short piece of glass tubing which is connected with the aspirating bottle by rubber tubing.

The air to be examined is drawn through the long tube and its bacterial or mould content is caught in the water. By plating 1 c.c. which would represent one-fifth of the total count for the amount of air aspirated we can easily calculate the content for a cubic meter.

In comparing the results with the aerobioscope with those obtained by exposing a plate as in Petri's method for ten instead of five minutes, it was found that the latter was sufficiently in accord to make it a satisfactory approximate quantitative method. The simplicity and ease of access to the colonies developing on it make it preferable when the air of operating-rooms or hospital wards is to be examined.

Of the fungi ordinarily obtained in examinations of the air the blue-green mould and the red yeast are the most common. *B. subtilis* and sarcina types of cocci are the most common bacterial colonies found upon exposed plates. Sewer air is as a rule free from bacteria, due probably to the fact that bacteria tend to adhere to moist surfaces. The importance of Flügge's droplet method of contamination of the air of a room is brought out in the discussion of infection with pneumonic plague. This is an important method in the transmission of tuberculosis.

CHAPTER XII

PRACTICAL METHODS IN IMMUNITY

THAT which prevents the gaining of a foothold by disease organisms in the animal body or which neutralizes their harmful products or destroys the parasites is termed immunity. In the main, the question of immunity hinges on the powers of resistance of the human body and the aggressiveness or virulence of the invading organism. It must always be kept in mind that immunity is only relative; thus the fowl, which is practically immune to tetanus, may be made to succumb by reducing its resistance by refrigeration or by increasing the amount of poison introduced. The insusceptibility which the fowl has to tetanus or which man has to many diseases of animals is best termed *inherent* or *inborn immunity*, and is at present only a subject of theoretical interest. When immunity to a given disease is obtained as a result of an attack of the disease in question or by laboratory methods of inoculation, this is termed properly an *acquired immunity*, and in the former case is a *naturally acquired immunity* and in the second an *artificially acquired immunity*.

Immunity then may be divided into that which is *inherent* and that which is *acquired*. Inherent immunity is such as is observed in the resistance of Algerian sheep to anthrax (ordinary sheep are very susceptible) or the fowl to tetanus and is of interest theoretically rather than practically. Acquired immunity may be brought about naturally as by an attack of a disease or artificially by laboratory measures.

As a result of an attack of a disease, which may be regarded as accidentally acquired, or in response to the stimulus of the injection of the organism or its products, we have developed in the man so infected or injected certain specific antagonistic properties to that organism, which are usually demonstrable in the blood-serum or other body fluids, and to which we apply the terms agglutinating power, precipitating power, opsonic power, or bacteriolytic power. The term *antibody* is also applied. All four powers may be present together in equal or in

varying degree or one or more may be absent. By agglutinating power we mean that which causes evenly distributed organisms to come together and form clumps. By precipitating power we mean the ability of such a serum to cause precipitates in a clear bouillon filtrate of the specific bacterium. Such antibodies are called precipitins or coagulins. By opsonic power we mean that antibody which so alters the resistance of bacteria that the phagocytes ingest them. By bacteriolytic power we mean that which brings about disintegration or lysis

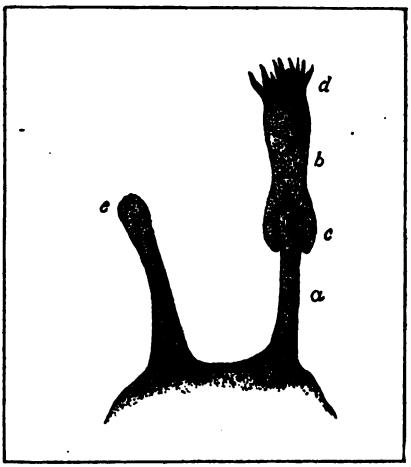


FIG. 47.—Receptors of the first order uniting with toxin. (*Journal of the American Medical Association*, 1905, p. 955.) *a*, Cell receptor; *b*, toxin molecule; *c*, haptophore of the toxin molecule; *d*, toxophore of the toxin molecule; *e*, haptophore of the cell receptor.

of the specific organism. The bacterium which causes the disease or which is used in inoculation for the production of immunity is termed the *specific organism.*

Artificially Acquired Immunity.—Of the different kinds of immunity only artificial immunity will be considered. This may be obtained in two ways: 1. By injecting the bacteria or their products into man or animals and as the result of the activity of the cells of the animal invaded, antibodies are formed which neutralize the toxins (antitoxins) or bring about lysis of the specific bacteria (bacteriolysins). These antibodies which are supposed to be thrown off (free receptors) from those body cells which have suitable fixation powers for the invad-

ing toxin molecule or bacterium may remain potential for months or years and so confer a more or less enduring immunity.

These fixation points are known as *cell receptors* and are intended for the assimilation of various foodstuffs by the cell. If destroyed by the toxin or bacterium they are reproduced in great excess by nature.

Not only may bacteria act in this way but foreign cells, such as red cells or various parenchymatous cells, when injected, give rise to antagonistic substances which act as factors in their destruction—hæmolysins for red cells, cytolysins for different parenchymatous cells. Such methods produce "*active immunity.*"

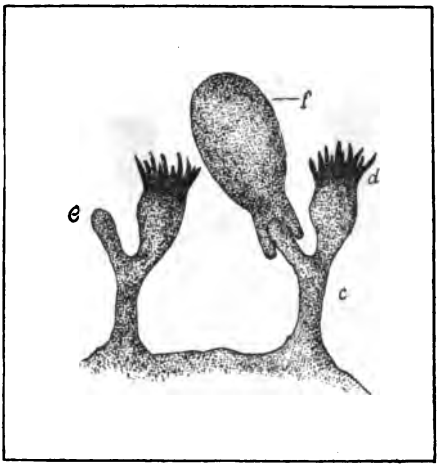


FIG. 48.—Receptors of the second order and of some substance uniting with one of them. (*Journal of the American Medical Association*, 1905, p. 1131.) *c*, Cell receptor of the second order; *d*, toxophore or zymophore group of the receptor; *e*, haptophore of the receptor; *f*, food substance or product of bacterial disintegration uniting with the haptophore of the cell receptor.

Antigen.—The substance which is injected and in reaction to which antibodies are produced is called an *antigen.*

2. When we take the serum of a man or animal immunized actively and inject it with its contained antibodies into a second animal or man, we confer an immunity on the second animal; but as his cells take no active part in the production of the immunity, but are only passive, we term this immunity "*passive immunity.*" If this serum which is introduced in passive immunity only neutralizes the toxic products of the infecting bacteria, we term it antitoxic passive immunity and designate the immune serum as *antitoxic serum.* If it destroys the organism, we call it *antimicrobic serum*, and the immunity, antimicrobic passive immunity. Some immune sera are both antitoxic and antimicrobic.

Toxins.—It is well to remember that some organisms produce a soluble or extracellular toxin which is given off while the bacterium is alive; and in other instances the toxin is intracellular and is only given off when the bacterium disintegrates; consequently, an antimicrobic serum may cause the liberation of toxin. Diphtheria, tetanus, or botulism antisera are instances of antitoxic sera, while practically all others are antimicrobic. The antidysentery serum against Shiga strains seems to have antitoxic power. *B. pyocyaneus* also has a soluble toxin.

Antitoxic sera.—There is but one factor to consider in an antitoxic serum and that is the protoplasmic particles which are thrown off from the cell in response to the

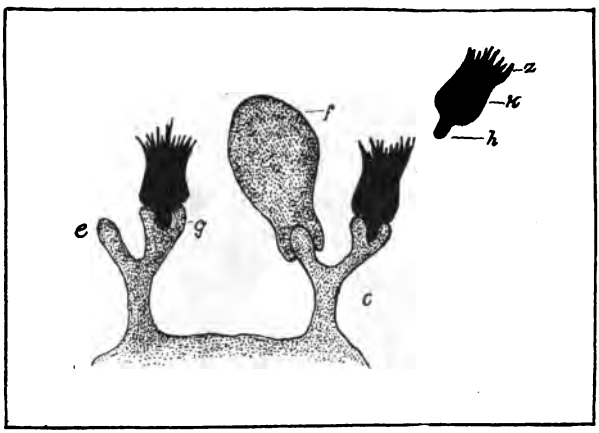


FIG. 49.—Receptor of third order, and of some substance uniting with one of them. (*Journal of the American Medical Association*, 1905, p. 1369.) *c*, Cell receptor of the third order—an amboceptor; *e*, one of the haptophores of the amboceptor, with which some food substance or product of bacterial disintegration (*f*) may unite; *g*, the other haptophore of the amboceptor with which complement may unite; *k*, complement; *h*, the haptophore; *z*, the zymotoxic group of complements.

injury incident to the attack upon the cell by the toxin particles. This free particle in the circulation represents the entire mechanism of antitoxic immunity. It is capable of uniting with the toxin molecule and neutralizing its toxic power, or rather so binding its combining end (haptophore group) that it is incapable of attaching itself to a cell, so that the poisonous end of the toxin (toxophore group) cannot have access to the cell.

The term toxin, strictly speaking, is applicable only to such bacterial poisons as (1) require a period of incubation before being capable of manifesting toxic symptoms and (2) can produce antitoxins.

For further discussion of toxins and antitoxins see under diphtheria, tetanus, botulism, and pyocyaneus infections.

Antimicrobic Sera.—In antimicrobic sera we have two factors to consider, the first is a protoplasmic particle quite similar to the antitoxin molecule, but which in itself has no power of injuring its specific bacterium. This particle is generally referred to as the *amboceptor* or *immune body*. It is the specific product of the activity of a specific bacterium or foreign cell against the body cells attacked. It withstands a temperature above 56°C. and of itself is incapable of injuring the bacterium in response to whose attack it was produced. The second factor in the bacteriolysis of the specific bacterium, or the hæmolysis of the specific foreign cell, is something normally present in the serum of every animal, and which is capable of disintegrating a foreign cell or bacterium, provided it can have access to the cell or bacterium through an intermediary amboceptor (hence the amboceptor is sometimes called an intermediary body). This something is called the *complement*. It is by some called alexine, by others cytase (Metchnikoff).

The complement cannot act upon and destroy an invading bacterium or cell unless the amboceptor is present to make the necessary connection. The complement is destroyed by a temperature of 56°C., so that, if we heat the serum from an immune animal to 56°C., the complement it naturally contains is destroyed, and the amboceptor it contains, which is not injured by such a temperature, is incapable of destroying bacteria or cells, unless we replace the complement which has been destroyed by fresh complement. This is done experimentally by adding the serum of a non-immunized animal which contains the complement, but no specific immune body (amboceptor), to the heated serum. This is termed "activating," and a serum so treated is said to be "*activated*." When an immune serum has been heated to 56°C., it is said to have been "*inactivated*."

Antimicrobic sera are not as efficient in treatment as antitoxic ones. It might be that if we could use homologous sera for treating man instead of the usual heterologous ones from the horse better results might obtain.

It would appear that a more hopeful outlook will obtain by combining serum therapy with chemo-therapy, thus a combination of antipneumococcic serum with sodium oleate seems capable of producing curative results which neither alone can bring about.

Again, a combination of vaccination (active immunization) with the injection of the antimicrobic serum (passive immunization) has been thought by some to be of value.

Sensitization.—When we allow a mixture of bacteria or cells to remain in contact with their specific immune serum which has been

inactivated, the amboceptors attach themselves to the bacteria or cells, so that now, upon adding normal serum (complement), these bacteria or cells are so prepared or mordanted that the complement can disintegrate them. This experiment of attaching amboceptors to cells is termed sensitizing and cells so treated are said to be *sensitized.*

Methods for Obtaining Immune Sera

While a convalescent from a disease may be utilized to obtain an antitoxic, agglutinating, opsonic, or bacteriolytic serum against the specific bacterium, yet this is more conveniently obtained from an animal which has been immunized against the bacterium or cell in question. The rabbit is the most convenient animal to employ for the production of immune sera where the object is to have at hand a serum for use in diagnosis.

Serum Diagnosis.—Where sera are used on an extensive scale, as in the production of curative sera, larger animals are employed. There are two applications of serum diagnosis: 1. Where the bacterium is known and the serum is to be diagnosed. 2. Where the serum is known and the bacterium is to be diagnosed.

The first is employed by testing the agglutinating or bacteriolytic power of the serum taken from a patient upon pure cultures of the organism which is suspected as the cause of the disease. The Widal test (agglutination) is the best instance of this procedure. This method is of practical value in the diagnosis only of typhoid, Malta fever, and para-typhoid. In diseases like cholera and bacillary dysentery, the disease has run its course before agglutinating power becomes apparent in the serum. This method, however, may be used to prove that a convalescent has suffered from a suspected disease. Thus, by testing the agglutinating power of a serum, one or two weeks after recovery from a suspicious case of ptomaine poisoning, we may be able to demonstrate that the case in question was cholera. The second method has wider application, and is the one in which we use the sera of animals which have been immunized with known bacteria. Organisms isolated from urine, fæces, or blood of patients, or those obtained from water or food supplies may be identified by testing the agglutinating, opsonic, or bacteriolytic power of known sera against them. This has a wide range of applicability. The testing of the opsonic power of the sera in man or animals immunized against plague, and possibly cerebrospinal meningitis, seems to give more definite information than do agglutination or bacteriolytic tests. With the majority of other organisms, however, the agglutination test is the one almost always preferred.

Even in a small laboratory there are no particular difficulties in the way of having on hand rabbits immunized against typhoid, paratyphoid, Malta fever, acid-producing and nonacid-producing strains of dysentery, cholera, etc. Just as we inject men with vaccines prepared from various bacteria in opsonic therapy, so we

inject animals to produce sera for diagnosis. We may use either a bouillon culture or the growth on agar slants taken up with salt solution as the inoculating material. This is heated for one hour at 60°C. to kill the bacteria. Where we desire to produce a serum which will disintegrate red blood cells (hæmolytic serum), we inject intravenously about 1 c.c. or intraperitoneally about 5 c.c. of the washed red cells of the animal for which we wish to produce a specific serum. For details see method of preparing hæmolytic amboceptor serum under Noguchi's modification of Wassermann test.

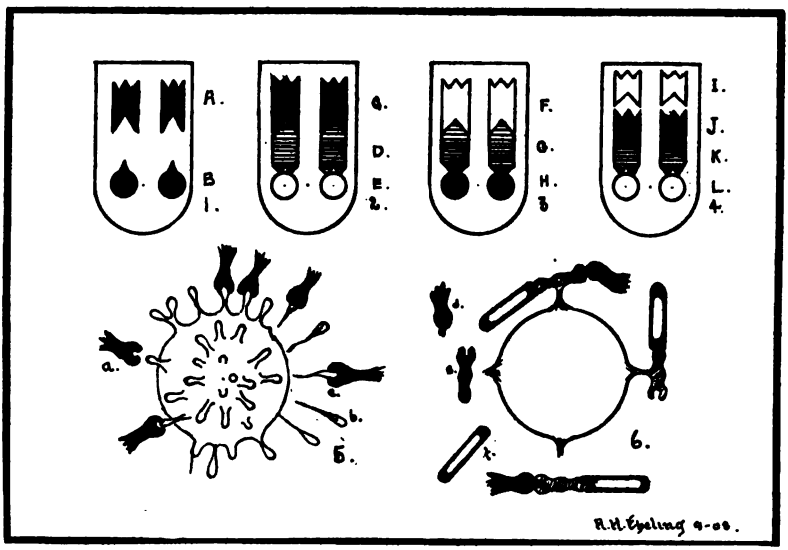


FIG. 50.—1, Red cells + normal serum. No amboceptor. No hæmolysis. A, Complement; B, normal red cell. 2, Red cells + immune serum. Complement and amboceptor. Hæmolysis. C, Complement; D, amboceptor; E, hæmolyzed red cell. 3, Red cells + immune serum heated to 56°C. Inactivated. Complement destroyed. No hæmolysis. F, Destroyed complement; G, amboceptor; H, red cells. 4, Red cells + heated immune serum + fresh serum. (Activated by contained complement.) Hæmolysis. I, Destroyed complement; J, fresh complement; K, amboceptor; L, hæmolyzed red cell. 5, Diagram showing antitoxin production. *a*, Toxin molecule; *b*, antitoxin molecule; *c*, neutralization of toxin by antitoxin. 6, Diagram showing bacteriolysin. *d*, Complement; *e*, amboceptor; *f*, bacillus.

Precipitating Sera.—For preparing a serum for the biological blood test we inject the rabbit intravenously with human serum in quantities of about 5 c.c. every fifth day. About one week after the last injection the antiserum obtained from the injected rabbit should be strong enough for $\frac{1}{10}$ c.c. to produce turbidity when added to 1 c.c. of a 1–1000 dilution of human serum in salt solution. Various controls are necessary when the test is used in medico-legal work.

Agglutinating Sera.—For obtaining an agglutinating or bacteriolytic serum for bacteria we inject about 1 c.c. of the killed bacterial bouillon culture subcutaneously or into the peritoneal cavity of the rabbit. The easiest way to inject the rabbit is to

hold the animal head down and plunge the needle in the median line into the abdominal cavity, forcing in the contents of the syringe. The intestines gravitate downward and by entering the needle below the limits of the bladder we avoid injuring any vital part. It may be more satisfactory to at first inject only about ½ c.c., and then if there is very little reaction, as shown by the appetite and spirits of the rabbit, to inject about four days later 1 c.c. About four or five injections at intervals of three to five days will usually produce an immune serum.

Injection of the antigenic material (blood cells, serum or bacterial emulsion) into the marginal ear vein may be employed. With this method, however, I have had several rabbits die in what was considered anaphylactic shock. (For the method of immunizing rabbits to produce a hæmolytic serum see Wassermann test.) In immunizing rabbits for the production of monovalent sera for type agglutination of meningococcus and pneumococcus we inject $\frac{1}{100}$ of the surface growth of a slant the first day, $\frac{1}{75}$ the second day and $\frac{1}{50}$ the third day. Allow a week to pass and then start in again with $\frac{1}{50}$ the first day, $\frac{1}{25}$ the second day and $\frac{1}{10}$ the third day. If the titre is not high enough one can give $\frac{1}{5}$ of a surface growth on the fourth day. These injections are of living organisms. The English inject killed cultures. Some animals do not seem to be capable of producing antibodies, so that it may be necessary to use one or more rabbits before a satisfactory serum is obtained. The most convenient way of obtaining serum for a test is to cut across one of the marginal veins of the rabbit's ear, and collect the blood in a Wright's U-tube. Centrifugalizing, we have the serum ready for use.

The vein can be made to stand out prominently by applying a compress dipped into very hot water. When a large amount of serum is desired it is better to use a test-tube with two pieces of glass tubing passing through a double perforated rubber stopper. To one of the projecting pieces of glass tubing a stout hypodermic needle is attached through the medium of 8 inches of rubber tubing and to the second piece of glass tubing passing through the stopper of the large test-tube another piece of rubber tubing is attached for suction. To obtain blood from the rabbit find the ensiform cartilage and insert the needle in the notch to the left and gently force it upward. Applying suction with the mouth the blood flows into the test-tube as soon as the needle enters the heart. By placing the tube of blood in the refrigerator the serum separates out from the clot. The removal of 20 to 30 c.c. of blood does not seem to affect the animals in the least and they can be used in this way time and time again. The immune body and agglutinin in serum remain active for weeks when kept in the refrigerator. Such sera may also be dried on paper as for amboceptor paper (Noguchi). The complement and opsonin, however, begin to deteriorate at once and have disappeared by the fifth day. Consequently, for opsonic and bacteriolytic and hæmolytic experiments, fresh serum—twelve to twenty-four hours—must be used, or it may be activated.

Agglutinins

While Gruber and Durham first noted that cultures of *B. coli* and *Sp. choleræ asiaticæ* would develop flake-like clumps, when treated with the specific serum, it remained for Widal to apply this agglutination reaction to the diagnosis of typhoid fever. In the production

of agglutination the bacterial or other cells give off *agglutinogen*, which acts on the lymphoid or possibly other cells of the animal injected with agglutinogen, to produce an antibody, with both haptophore and agglutinophore receptors, which is called *agglutinin*. Agglutinins are more resistant than complement, withstanding a temperature of 60°C. for thirty minutes, while the complement of a bacteriolysin is destroyed at 55°C. Therefore in the inactivation of serum we do not destroy agglutinins.

If the temperature is raised above 60°C. the agglutinophore molecule is destroyed but the haptophore group is intact and such a damaged agglutinin can combine with its specific bacterium without, however, causing agglutination. To this we apply the name *agglutinoid*.

Usually a serum shows stronger agglutination when concentrated than when more diluted. We do observe reactions, however, in which the lower dilutions fail to agglutinate while the higher ones give agglutination. This is supposed to be caused by the presence of agglutinoids which have stronger combining power than agglutinins. In such cases we call these agglutinoids *pro-agglutinoids* and state that the paradoxical reaction is caused by the presence of pro-agglutinoids which must be absorbed before the agglutinins can cause clumping. The designation for the concentration in which agglutination fails to occur is designated the *pro-agglutinoid zone*.

An immune serum will often agglutinate organisms closely related to its specific organism in higher dilutions than would a normal serum. Such agglutinins are called *group agglutinins* and their power is always markedly lower than the specific agglutinins.

It has been found that a serum treated with emulsions of its specific organism loses all its agglutinating power for that organism, and, along with this, for closely allied bacteria which it was previously capable of agglutinating in low dilutions. If, however, this serum is treated with one of the allied bacteria, instead of the specific one, the agglutinating power for the specific organism is but little affected. These reactions are spoken of as *agglutinin absorption* methods and are of value as showing whether an infection is a mixed one or only due to a single one of a number of organisms related from the standpoint of group agglutination. The agglutination for the specific organism or organisms is always much higher than for group agglutinin organisms. See Dreyer method. Again, if an unknown organism removes all agglutinins from an immune serum we known that it is the same organism as the specific one, but if it only removes group agglutinins, leaving the specific agglutinins unbound, it is proven to be only a group organism. This method is probably the most exact one for determining the relationship of bacteria.

This is the best method for determining the different groups or strains of the meningococcus. At present we regard these meningococcus groups as being the same organism, although further study may show them to occupy the same relationship to one another as do the typhoid and paratyphoid organisms.

There are two methods of testing the agglutinating power of a serum—the microscopical and the macroscopical or sedimentation method.

The Widal Reaction.—1. For the microscopical method draw up serum to the mark 0.5 of the white pipette. Then draw up salt solution to the mark 11. This when mixed gives a dilution of 1 to 20. (It is more convenient to make the serum dilutions with a graduated rubber bulb capillary pipette.) One loopful of the diluted serum and one loopful of a bouillon culture or salt solution suspension of the organism to be tested gives a dilution of 1 to 40. One loopful of the 1 to 20 diluted serum and 3 loopfuls of the bacterial suspension give a dilution of 1 to 80. These two dilutions answer in ordinary diagnostic tests. The red pipette with a 1 to 100 to 1 to 200 dilution may be used where dilutions approaching 1 to 1000 are desired. Having mixed the diluted serum and the bacterial suspension on a cover-glass, we invert it over a vaselined concave slide and examine with a high power, a dry objective (1/6 inch). It is neater to press down the vaselined periphery of the concavity on the cover-glass. This sticks to the borders of the cover-glass and the preparation is easily handled. It is simpler to make a ring of vaseline to fit the cover-glass and make the mixture of diluted serum and culture in the center of this ring or square. Then apply the cover-glass, press it down on the vaseline ring and examine as with the ordinary hanging drop. In making dilutions it is preferable to use salt solution, as the *phenomenon of agglutination requires the presence of salts.* Ordinarily, thirty minutes is a sufficient time to wait before reporting the absence of agglutination. Agglutination is more rapid at body temperature than at room temperature. In reporting agglutination, always give time and dilution. It is absolutely necessary that a control preparation be prepared in every instance; that is, one with the bacterial culture alone or with a normal serum of the same dilution as the lowest used. Some normal sera will agglutinate in 1 to 10 dilution, and *group agglutinations* (as paratyphoid with typhoid serum) may occur in 1 to 40 or possibly higher. It is very unusual for sera to agglutinate any other bacteria than the specific one in dilutions as high as 1 to 80.

Macroscopic Agglutination.—2. For the macroscopical or sedimentation test, take a series of small test-tubes (3/8 × 3 inches) and deposit 1 c.c. of salt solution in each of the series. Now, having taken an empty test-tube, drop 4 drops of serum in it and then add 12 drops of salt solution. This approximately gives 1 c.c. of a 1 to 4 dilution of the serum. It is more exact to make the 1 to 4 dilution with a graduated pipette. With a rubber-bulb capillary pipette, which has been graduated to hold 16 drops or 1 c.c., draw up the contents of the tube containing the 1 to 4 serum and add it to the next tube containing 1 c.c. of salt solution. This gives 2 c.c. of a dilution of 1 to 8. Now mix thoroughly by drawing up and forcing out with the bulb pipette, and then withdraw 1 c.c. and add to the next tube containing 1 c.c. of salt solution. This gives a dilution of 1 to 16. Having mixed as before, again withdraw 1 c.c. of the mixture and add it to the 1 c.c. in the next tube. We now have a dilution of 1 to 32. Again withdrawing 1 c.c. and adding it to the fourth tube containing 1 c.c. of salt solution we have a dilution of 1 to 64. In tube 1 there is now 1 c.c. of a dilution of the serum of 1 to 8; in tube 2, there is 1 c.c. of a dilution of 1 to 16; in tube 3 of 1 to

32. Tube 4 contains 2 c.c. of 1 to 64. The dilutions can be carried on in the same manner to any extent that may be desirable. In cholera agglutinations we may run up to 1 to 5000 or thereabouts. Of course, where such dilutions are employed, we generally start with 2 c.c. of 1 to 50 in the first tube. When we have completed the series, each tube having 1 c.c. of diluted serum, and the last 2 c.c., we remove with the pipette 1 c.c. from the last tube and discard it by ejection from the pipette leaving 1 c.c. in the last tube. Now adding 1 c.c. of a culture of typhoid or any other organism, we have the dilution of the serum in each tube doubled. Tube 1 now contains a serum in dilution of 1 to 16, acting on the bacteria; tube 2 of a 1 to 32; tube 3 of a 1 to 64. Now place these tubes in the incubator and, after two to five hours or overnight, we examine for the clearing up of the supernatant fluid. If the serum in a certain dilution agglutinates, the clumps gravitate to the bottom and the upper part becomes clear. If so desired, these dilutions may be carried on to 1 to several hundred in the same way. It is safer to work with dead cultures instead of living ones. To prepare, take a twenty-four-hour agar slant culture of typhoid or paratyphoid and emulsify in salt solution (about 6 c.c. to a slant).

By adding 0.1 of 1% of formalin to the typhoid emulsion and placing in the ice-box the cultures will be found sterile in about three days. The emulsion should be shaken twice daily while undergoing sterilization in the ice-box. Such cultures are not easily contaminated and appear to retain their agglutinable qualities for several months. The macroscopic methods are preferable with such dead cultures.

A very convenient method in general use in Germany is the following: Make dilutions of serum in ordinary test-tubes ($\frac{3}{4} \times 6$ inches) as described for the small test-tubes.

Then take a loopful (2 mg.) of culture from an eighteen to twenty-four-hour-old agar culture and emulsify it thoroughly in the dilution in the first test-tube—repeat the process in the second tube and so on. This procedure is much safer than when live cultures are added with a pipette. Again, the dilution is unchanged by this addition whereas it is doubled when an equal volume of culture is added to the diluted serum. A control should always be made in normal salt solution. After incubating, observe flocculent precipitates (agglutination) by tilting the fluid in the tubes to form a thin layer and to obtain the most advantageous light and look for a fine curdy precipitate (agglutination) or a uniformly turbid emulsion (negative reaction).

The method of using a slide with two vaselined rings, one containing an emulsion in the specific serum and the other in salt solution is of great practical value. This method is described under cholera.

Oxford University Standard Agglutination Methods (Dreyer).—In order to have a standard method for comparison of agglutination results following vaccination with typhoid or paratyphoid vaccines, as well as for the diagnosis of a typhoid like infection arising in a vaccinated person the Dreyer method is generally recommended.

At the Army Medical School they have made some slight modifications in the original technic which would appear to simplify the method.

The method consists simply of macroscopic agglutination tests on varying dilutions of serum, using a killed culture of *B. typhosus* or *B. paratyphosus* A or B, the agglutinability of which has been determined by titration against a given standard, established by the Department of Pathology, University of Oxford.

The necessary apparatus is readily available, and consists of a Wright's capsule, for securing blood; about thirty small test-tubes, chemically clean, but not necessarily sterile; one 1-c.c. pipette, graduated in tenths, such as used in Wassermann work; one 2-c.c. pipette for making the dilutions; a few ounces of physiologic sodium chlorid solution, not necessarily sterile; and a quantity of the standard culture. By comparison with this standard culture, a new supply may be made for extended work.

The technic for the test is quite simple. Sufficient blood is taken in the capsule to yield 0.3 c.c. of serum. This amount is withdrawn in a pipette and transferred to the first tube. This tube has previously received 2.7 c.c. of saline solution, while the following nine tubes have each received 1.5 c.c. of saline solution. Of this first 1 to 10 dilution of serum, 1.5 c.c. are carried into the second tube, mixed thoroughly, and 1.5 c.c. of this 1 to 20 dilution is carried forward. This procedure is carried out through the series of ten tubes, the last 1.5 c.c. being discarded. Beginning in the weaker dilution, the diluted serums are each divided into three tubes, each tube containing 0.5 c.c. of the serum dilution. The tube rack then contains three series of ten tubes containing serum dilutions ranging from 1 to 10 to 1 to 5120.

To each tube in the first series, 0.75 c.c. of the standard culture of *B. typhosus* is added; to each tube of the second series, 0.75 c.c. of the standard culture of *B. paratyphosus* A is added; and to each tube of the third series, a like amount of the culture of *B. paratyphosus* B is added. The tubes are then thoroughly shaken.

The tubes are then placed in a water bath at from 50 to 55°C. for two hours; are removed and cooled for fifteen minutes, and are then read. The highest dilution showing agglutination, without sedimentation visible to the naked eye, gives the reading. The dilution of the serum in this tube, divided by the factor of agglutinability of the culture used, gives a final reading expressed in the number of agglutinin units per cubic centimeter of the serum. (It must be kept in mind that the addition of the culture has increased the dilution of the serum one and a half times, the first tube representing a dilution of 1 to 25, the last a dilution of 1 to 12,800). These readings are comparable with each other, whenever the same technic has been used, and a standard agglutinable culture has been used.

If it becomes necessary to make up a new supply of the standard agglutinable culture of *B. typhosus*, the procedure is as follows: *B. typhosus* is subcultured daily in broth, for about ten days, to increase its agglutinability and reduce its autoagglutinability. Finally it is planted in broth in partly filled flasks and incubated twenty-four hours. At the end of that time, 0.1% formaldehyd solution (full strength) is added, it is placed in an ice box for four or five days, and it is shaken repeatedly. The then sterile culture is ready to be standardized for (1) opacity and (2) agglutinability.

To standardize for opacity, two series of fifteen tubes each (tubes of equal size and of clear glass) are set up, and varying dilutions of standard culture in one series,

and the culture under standardization in the other, are made in accordance with the accompanying schedule.

Dilutions

Culture—

0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0 1.2 1.4 1.6 1.8 2.0

Physiologic sodium chlorid solution—

1.9 1.8 1.7 1.6 1.5 1.4 1.3 1.2 1.1 1.0 0.8 0.6 0.4 0.2 0.0

A tube from one series, chosen at random, is matched for opacity with the tubes in the other series. The dilution of each culture in these tubes is noted. This procedure is repeated six times and the average taken. The necessary dilution of the new culture is thus determined, the dilution to be made with physiologic sodium chlorid solution, to which has been added 0.1% formaldehyd solution. It is then to be bottled and kept in the ice-box.

To standardize for agglutinability, an immune serum is procured. A rabbit immune serum of known agglutinin unit content is to be preferred, though human serum, taken soon after inoculation, serves the purpose. The agglutinating serum is set up in two parallel series, in varying dilutions, the variations not to be excessive. To one series the standard culture is added, to the other the culture under standardization. These tubes are then shaken, incubated at 55°C. for two hours and read for the highest dilutions showing agglutination visible to the naked eye. That dilution in the known culture is to that dilution in the new culture as the factor of the known culture is to x (the factor of the new culture).

In noninoculated persons who have not had typhoid (or paratyphoid) fever, agglutination in a dilution of 1 in 25 justifies a strong suspicion of typhoid (or paratyphoid) infection. But the test must be applied again in the course of a few days to ascertain whether there is any change in the titre of agglutination. Marked agglutination in a dilution of 1 in 50 or more is (nearly always) diagnostic of active typhoid (or paratyphoid) infection.

A *noninoculated* "carrier" will normally show no important change in the titre of his serum on repeated examination at short intervals.

Inoculated persons if quite recently inoculated will usually show a high titre of specific agglutination. A rapid rise in titre sets in within two to four days of inoculation. This is followed by a fall at first rapid, but subsequently becoming very slow, so that a relatively high titre is maintained for a long period (even for years). During this period examinations made at intervals of a few days give practically identical readings.

It follows that in the case of inoculated persons the diagnosis of active typhoid (or paratyphoid) infection will require two or more successive examinations of the serum.

(*a*) If the individual is suffering from active *typhoid* infection his titre of typhoid agglutination will exhibit the usual rise and subsequent regular fall seen in noninoculated subjects, but starting from and returning toward the higher base line of inoculated persons.

(*b*) If the individual is suffering from active *paratyphoid* infection one of three things may occur as regards his *typhoid* agglutination titre, namely:

1. No appreciable change may occur in the titre of typhoid agglutination.
2. A relatively slight rise may occur, followed by a fall toward the former level.
3. A marked rise may occur synchronous with the rise in paratyphoid agglutination titre, and subsequently followed by the usual fall toward the former level.

Meanwhile the titre of *paratyphoid* agglutination runs the normal course of rapid rise to a maximum (usually exceeding the maximum typhoid titre) followed by a fall, at first rapid and then slower as already described for typhoid subjects, and falling *below* the persistent base line of typhoid agglutination of inoculated persons.

In the case of **mixed infections** whether in inoculated or noninoculated persons the agglutinin curves for the different infecting organisms are usually not synchronous, and they pursue their ordinary course independently of each other.

Agglutination of Meningococci.—For diagnosis of a colony on a plate made from a carrier inoculate a slant of blood or serum agar from the colony, which will furnish abundant growth for the macroscopic dilutions to be made the next day. Prepare a 1 to 100 dilution of a polyvalent serum and emulsify in this serum enough of the growth to give a distinct turbidity.

Another tube (9×1 cm.) containing 1 to 50 normal horse serum, should be treated in the same manner. These tubes should be incubated at 55°C. for sixteen hours or over night.

Some prefer a 1 to 200 dilution of the polyvalent serum. Certain meningococcus-like organisms agglutinate in the horse serum as well as the immune serum.

In testing for types we take several tubes with dilutions of the monovalent sera running from 1 to 100 to 1 to 1600. It is not unusual to obtain agglutinations of 1 to 800 or 1 to 1600 with the specific organism.

Houghton has modified the slide agglutination method of Krumwiede as follows:

Take a large loopful of 1 to 10 normal horse serum. Emulsify the colony from a plate in this serum. If agglutination occurs it is not a meningococcus. If agglutination does not occur he rubs in a small loopful of polyvalent serum (undiluted). The curdy clumping begins to show itself within a minute or so and is distinct with the naked eye, or better with a magnifying lens, or the ⅔-inch objective. After the preparation dries do a Gram stain to verify the clumped organisms.

By successively using small loopfuls of type sera, before adding the polyvalent, one can quickly obtain evidence as to type to be later verified by macroscopic agglutination. (Along this line one can do slide agglutinations for pneumococcus types.)

Agglutination of Pneumococci for Type Determination.—Take a specimen of the albuminous pneumonic sputum and do a Gram's stain. From this the type III organism may be determined by its large capsule. Inoculate a well-washed, bean-sized piece of sputum, emulsified in sterile saline, into the peritoneal cavity of a mouse. The mouse usually shows marked signs of illness in six to eight hours. Remove a small drop of the peritoneal exudate with a capillary pipette and examine to note abundance of pneumococci. In such case kill the mouse and wash out the peritoneal cavity with 4 to 5 c.c. of saline. Collect these washings in a centrifuge tube and centrifuge at a low speed to throw down cells and fibrin.

The supernatant bacterial suspension is then removed and transferred to a second centrifuge tube and centrifuged at high speed. Remove the supernatant fluid and emulsify the sediment in sufficient saline to make a moderately heavy suspension (about equal to an eighteen-hour bouillon culture of pneumococcus). Mix 0.5 c.c. of the emulsion with an equal quantity of the diagnostic sera and incubate for one hour at 37°C.

Pneumococcus suspension 0.5 c.c.	Serum I (1 : 20) 0.5 c.c.	Serum II (undiluted) 0.5 c.c.	Serum II (1 : 20 0.5 c.c.	Serum III (1 : 5) 0.5 c.c.
Type I.....	++	−	−	−
Type II.....	−	++	++	−
Subgroups IIa, b, x.....	−	+	−	−
Type III.....	−	−	−	++
Type IV.....	−	−	−	−

NOTE.—For agglutination of red cells see Blood Transfusion Tests.

PRECIPITIN REACTIONS

In the diagnosis of bacterial infections the agglutinating tests are so much more satisfactory that precipitin tests are rarely applied. As will be noted under the *Meningococcus* such a test has been recommended for the diagnosis of cerebrospinal fever.

In the technic of precipitin reactions for bacteria one filters two or three weeks' bouillon cultures of a given organism through a Berkefeld filter. (*Precipitinogen.*) The filtrate should not only be perfectly transparent but also sterile as subsequent bacterial growth would give turbidity similar to a positive reaction. The *precipitin* containing serum is prepared by injecting rabbits intravenously with bacterial filtrates as prepared above or with the bacteria themselves. The methods are similar to those for preparing agglutinating or hæmolyzing sera.

Test: To four tubes each containing 2 c.c. of the bacterial filtrate are added increasing quantities of the serum to be tested; 0.05 c.c. to the first tube, 0.1 c.c. to the second and 0.5 c.c. to the third, and 1 c.c. to the fourth. Controls of positive and negative precipitating sera should also be prepared. The tubes are not shaken and the reaction should be allowed five or six hours at room temperature before final readings are made. When the serum is strongly precipitating the clouding of the clear fluid should take place in ten to twenty minutes.

In the *biological blood test* rabbits are immunized intravenously either with whole blood taken in citrated salt or with serum alone. For class work I use the blood of a chicken injected intravenously into a rabbit. An immune serum thus prepared contains hæmolysins as well as precipitins. The hæmolyzing effect of such a serum on the nucleated fowl's red cells shows well when examined in a hanging drop. The bleeding of the rabbit should be done after a period of fasting to avoid any opalescence of the serum.

Precipitating sera should be kept in the cold and may have one-tenth of 1% carbolic acid added as a preservative, or they may be preserved on paper strips.

The suspected blood stain should be extracted with normal salt solution and should be filtered until perfectly clear. An approximate strength of 1 to 1000 of the blood is desirable. This can be estimated as given under albumin in urine, with the U-shape tubing.

Test: Place 2 c.c. of the 1 to 1000 extract of the stain to be examined in tube 1, 2 c.c. of 1 to 5000 in tube 2, and 2 c.c. of 1 to 10,000 in tube 3, adding to each tube 0.1 c.c. of the precipitating serum.

In another tube put 2 c.c. of a 1 to 1000 salt solution dilution of the serum of the animal from which the suspected blood is supposed to come and add 0.1 c.c. of precipitating serum.

Various other controls as with normal rabbit serum, etc., are necessary for medico-legal application.

The tubes should not be shaken and may be kept at room temperature or in the incubator. A positive reaction appears in two or three minutes as a clouding at the bottom of the tube which becomes a distinct precipitate in fifteen or twenty minutes. Readings should be made at the end of twenty minutes as reactions occurring subsequently have no significance.

Precipitin Tests for Pneumococci.—A very valuable method is to add 0.5 c.c. of very clear urine from a case of pneumonia to an equal amount of the three-type sera.

A positive precipitin reaction shows as a faint cloud or heavy flocculent precipitate. The reaction is present in about 65% of cases due to Type I, II or III.

The precipitating substance may appear in the urine as early as twelve hours after the initial chill.

Krumwiede takes an albuminous specimen of sputum in a small beaker (if the sputum is very mucoid the test is not satisfactory in

our hands) and heats it in a water bath to coagulate the albumin. The coagulum is then rubbed up with about 1 c.c. of saline in the water bath for five minutes. The saline with the soluble pneumococcus precipitating substances is then centrifuged to clarify and 0.2 c.c. of this extract is deposited on the surface of 0.2 c.c. of the three-type sera and a cloud at the junction of the fluids (ring test) shows to which group the case belongs.

Vincent has recommended a precipitin test for epidemic cerebrospinal meningitis which has the advantages of being simple and more immediate than cultures and of particular value in those cases when meningococci cannot be found in the smears or in cultures from the cerebrospinal fluid. It is performed by adding 1 or 2 drops of antimeningococcic serum to a tube of fresh cerebrospinal fluid which has been cleared by centrifugalization for ten to fifteen minutes. After adding the serum the tube is placed in the incubator at 52°C. for two to five hours together with a control tube. The formation of a precipitate (turbidity) shows a positive test.

Avery's Method for Grouping the Pneumococcus (Artificial Mouse).—I. Meat infusion broth, 0.3 to 0.5 acid to phenolphthalein. This should not be sterilized under pressure.

II. Prepare also a sterile 20% solution of dextrose and a flask of defibrinated rabbit blood.

III. Every 100 c.c. of medium should contain 90% of the above broth and 5% each of dextrose and rabbit blood.

The medium is tubed in 4 c.c. quantities and should not be reheated.

Care should be taken to obtain a specimen of sputum from the deep air passages and to avoid mouth secretions.

A portion the size of a bean should be selected and washed even more carefully than for mouse inoculation, passing it 3 or 4 times through sterile salt solution.

The sputum is then ground in a sterile mortar, adding ½ to 1 c.c. of broth drop by drop. This emulsion is then introduced directly into the medium.

After inoculation, the tubes are incubated for five hours at 37°C. A smear is then made, stained by Gram, and a blood agar plate is inoculated.

The tube is now centrifuged at low speed for two minutes, just enough to throw down the red cells but not enough to bring down the bacteria, and the supernatant fluid is transferred into a second tube.

There are two methods of proceeding from this point.

I. *The Precipitin Method.*—To the above supernatant fluid add 1 c.c. of sterile bile and place the tube in water bath at 37°C. for twenty minutes. If not clear at the end of this time, centrifuge. The clear fluid is then used for a precipitin test, according to the Blake method as follows:

Tubes are set up in following manner:

Tube 1	0.5 c.c. Serum I (1 to 10)	plus 0.5 c.c. culture.
Tube 2	0.5 c.c. Serum II (undiluted)	plus 0.5 c.c. culture.
Tube 3	0.5 c.c. Serum II (1 to 10)	plus 0.5 c.c. culture.
Tube 4	0.5 c.c. Serum III (1 to 5)	plus 0.5 c.c. culture.

An immediate specific precipitin reaction occurs in the tube containing homologous immune serum. Incubation is usually not necessary. A precipitate in tube 2 and not in tube 3 indicates Subgroup II.

II. If bile is not at hand, an agglutination test may be made directly on the fluid after removal of the red cells.

The growth of the pneumococcus in this medium is not dependent upon virulence, as in the mouse, therefore, greater care must be taken to avoid contamination by mouth types, by careful selection and washing of the sputum.

Deviation of the Complement

It has been found that if there is not sufficient immune body in a mixture of normal serum, containing abundant complement, and bacterial emulsion, only a portion of the bacteria will be destroyed. Increasing the amount of immune body with a constant quantity of normal serum, we reach a point where all the bacteria are destroyed. Now, if we continue to increase beyond this point the addition of immune serum, the destruction of the bacteria ceases, and the cultures will again contain myriads of living bacteria (*Neisser-Wechsberg Phenomenon*).

To carry out the test, make a series of tubes containing mixtures of bacteria with the same quantity in each of normal serum. Thus, each tube contains ½ c.c. of bacterial emulsion and ½ c.c. of 1 to 10 normal serum. Now inactivate a tube of 1 to 100 immune serum and to each of the tubes of normal serum and bacterial emulsion add increasing drops of the inactivated 1 to 100 immune serum. Thus, 1 drop to No. 1 tube, 2 drops to No. 2 tube, and so on. After incubating for two hours, we take a pipette and plate out a fraction of a drop in an agar plate. The limit at which bacteriolysis is complete is shown by there being an absence of colonies.

Beyond or below that point colonies are more or less abundant. The explanation of this phenomenon of deviation or deflection of the complement is that where we have an excess of amboceptors for available receptors on the bacterial cells, only a portion of the amboceptors can attach themselves to their specific bacteria. The free amboceptors, not being able to form a union with the bacterial cell receptors (for which they have a greater affinity), combine with the complement present. Unless the complement be in excess, there will be no free complement left to join on to the amboceptors attached to the bacterial cells, and consequently bacteriolysis does not take place and the plate cultures show an abundance of colonies.

Stimson has found, in titrating his complement and amboceptor for complement-fixation tests, that keeping his complement content constant and successively increasing the amount of amboceptor gives increasing hæmolyzing effect up to a certain point, beyond which the further addition of amboceptor causes a lessening of hæmolytic power.

This he regards as due to deviation of complement and in his tests he prefers to keep a fixed amount of amboceptor and adjust his titrations by increasing complement rather than amboceptor.

Fixation or Absorption of the Complement

One of the controversies in connection with the nature of the complement is that regarding the question of the unity of complements or whether there exist different kinds of complements for different amboceptors (unity and multiplicity of complement). To prove that a single complement will act with varying amboceptors, Bordet and Gengou showed that the same complement would unite with both hæmolytic and bacteriolytic immune bodies. If to a mixture of typhoid bacteria and inactivated typhoid immune serum some guinea-pig serum is added and the mixture allowed to remain at 37°C. for two hours, and then sensitized red cells be added and the mixture again placed in the incubator for two hours, no hæmolysis will be found to have occurred, because the bacteria have absorbed all the guinea-pig complement through the intervening typhoid amboceptors, and there is no complement left to hæmolyze the red cells through the specific red blood-cell amboceptors.

If, instead of immune typhoid serum, the serum of a normal person had been used, there would have been no amboceptors to unite the complement to the bacterial cells. The complement would then be at hand to unite with the sensitized red cells subsequently added and bring about their hæmolysis, as shown by the ruby red color of the fluid.

This *phenomenon of Bordet and Gengou* has been utilized by Wassermann for the diagnosis of diseases where cultures are not applicable. It is well recognized, however, that the body in a syphilitic serum which reacts with the antigen is not an amboceptor but a lipoidophilic substance, which has the property of linking complement to the lipoidal antigen. The name *reagine* has been proposed for this lipoidophilic substance. A similar substance is present in the serum of *yaws* cases and the Wassermann reaction is just as constant in such cases as in syphilis.

It is in the diagnosis of syphilis that it is best known. It having, until recently, been impossible to obtain cultures of *Treponema pallidum*, we use an emulsion of the liver of a syphilitic fœtus, which has been filtered so as to be clear, instead of a culture. The syphilitic liver, as can be observed by staining according to Levaditi's method, is packed with spirochætes.

Antigen.—While Noguchi has recently obtained pure cultures of the organism of syphilis yet the antigen prepared from such cultures was not found as satisfactory by Craig and Nichols as that from the liver of a syphilitic fœtus, cases of syphilis which showed strongly positive tests with ordinary antigen not giving a positive test with the specific antigen.

It has now been found that lecithin or, preferably, emulsions of various normal organs may be substituted as antigen for the syphilitic liver, *the antigenic power being due to lipoids*. Aqueous extracts contain in addition to lipoids, substances which render the antigen unstable—alcoholic extracts are more stable and contain less anticomplement. The preparation of acetone insoluble antigen is described under Noguchi's method and that of syphilitic liver under the Wassermann reaction.

Many prefer to use *cholesterinized antigen*. For its preparation:

Prepare guinea-pig, beef or human heart muscle as described under acetone insoluble antigen and extract 50 grams of this finely cut up muscle with 500 c.c., absolute alcohol for two weeks at 37°C. Then filter and add to one-half this filtrate about 7 grams of C. P. cholesterin. Keep in 37°C. incubator over night and then keep at a temperature of 16°C. for three hours. This precipitates excess of cholesterin. Filter and to the filtrate add the other one-half of the heart extract, giving an antigen half saturated.

In our laboratory we have found this antigen rather sensitive and not altogether reliable and prefer to use the acetone insoluble one of Noguchi.

Reagine.—For the immune bodies or rather the reagine we take the serum of the patient, or if a case of locomotor ataxia or general paresis, the cerebrospinal fluid.

In using cerebrospinal fluid it is customary to employ 0.1 c.c., 0.2 c.c. and 1 c.c. quantities instead of the amounts given for blood-serum as directed in the tests to follow.

It is usual to expect strong fixation with a paresis fluid in the smallest amount noted above. For tabes use more fluid, as 0.5 and 1 c.c.

Noguchi's Modification of the Wassermann

For the *suspension of red cells* use a ½% suspension of washed human red cells.

For *complement* use fresh guinea-pig serum in a dilution of 1 part to 1½ parts of salt solution (40%).

Should it be impossible to secure guinea-pig serum one may use human serum from a man known to give a negative Wassermann. Make a 40% solution of this serum and to each of 5 or 6 test-tubes, containing each 1 c.c. of ½% emulsion of red cells and 1 unit of amboceptor paper, add varying amounts of the diluted human serum: 0.05, 0.07, 0.09, 0.1, 0.15 and 0.2 c.c. The tube which on incubating ½ hour in water bath at 37°C. shows complete hæmolysis contains 1 unit of complement. For the test we use 2 units of amboceptor and 1 unit of complement.

The guinea-pig complement may be titrated in the same way. It is well to make this preliminary titration along with that for amboceptor, as given below, before making the Wassermann tests.

Method.—Take four small test-tubes (12 by 125 mm.) label *1a*, *1b* and *2a*, *2b*, respectively. Into *1a* and *1b* each put 1 drop of the serum of the patient to be tested and into *2a* and *2b* each put 1 equal size drop of the serum of a person known to give a positive test for syphilis. The small drop delivered by a very finely drawn-out capillary pipette, held vertically, is equal to about 0.02 c.c. or 50 drops to 1 c.c. Next add to each of all four tubes 1 c.c. of the ½% suspension of washed red cells. Then add to each tube 0.1 c.c. of the 40% fresh guinea-pig serum. Now add to tube *1a* and tube *2a* each 0.1 c.c. of the 1 to 10 antigen dilution (opalescent working antigen emulsion). Tubes *1b* and *2b* are controls not containing antigen. Mix contents of tubes thoroughly and incubate at 37°C. for one hour or for one-half hour in a water-bath. Now add to each of the four tubes 2 units of the immune hæmolytic serum, as measured off on the amboceptor paper strip—thus with a paper of which 2 mm. equals 1 unit, drop into each tube 4 mm. of the strip.

The tubes without antigen (*1b* and *2b*) should show good hæmolysis. Tube *2a*, that of the known syphilitic, with antigen, should not show hæmolysis and that of the person examined (*a*) should show hæmolysis in case the test is negative for syphilis. Moderately positive cases may show a slight trace of hæmolysis. Where the tubes without antigen are without colour (no hæmolysis) it shows that there is some anticomplementary factor at work and that the tests should be regarded as unsatisfactory.

This failure to hæmolyze in the control tubes may be due to anticomplementary substances in the serum or to the bacterial contamination of the serum, the proteids of the disintegrating bacteria possibly preventing hæmolysis by antigenic action and thus absorbing the complement, which otherwise would bring about hæmolysis.

It is advisable also to employ the serum of a person known to be free from syphilis. In this case we should use two additional tubes, *3a* and *3b*, conducting the test as for the syphilitic control serum. Where a number of sera are to be examined we take additional tubes *4a*, *4b*; *5a*, *5b* and so on.

If a normal serum is not used then use a *system control* in which there are put all the reagents noted above except the human sera from the patient or the syphilitic control.

When examining a large number of sera at the same time it is well to use rubber adhesive plaster labels with the name of the patient written on it as well as the number. The greatest care should be exercised to avoid mistakes in numbers.

The majority of workers prefer to use inactivated serum for the test. In this case we should add four times as much of the inactivated serum as for the unheated serum (0.08 instead of 0.02).

Inactivation not only destroys complement but likewise diminishes the strength of the *reagine* content of the serum. Factors such as character of food and general condition influence the complement strength of guinea-pig serum so that it is advisable to titrate the guinea-pig serum. To do this take 1 c.c. of a ½% emulsion of human red cells and drop in 1 unit of amboceptor paper. The amount of complement which will entirely hæmolize the red cells in one-half an hour in water-bath equals 1 unit of complement.

We prefer to use a single unit of complement and 2 units of amboceptor, thus doubling the amboceptor unit. Stimson has found, however, that *increasing amboceptor* content may cause a *deviation of complement*, so that he prefers to use a single unit of amboceptor and 2 units of complement. In the original Wassermann technic the units of both complement and amboceptor are doubled. In the Noguchi only the amboceptor unit is doubled. Before making the Wassermann tests we take a series of 5 or 6 test-tubes, each containing 1 c.c. of a ½% suspension of red cells and 0.1 c.c. of 40% dilution of guinea-pig serum. Into the first tube put a piece of amboceptor paper representing ½ unit of the previously standardized paper. Into the second tube drop in a piece of paper equal to 1 unit. One and one-half units are dropped into the third tube, two units into the fourth tube, two and one-half into the fifth one and three units into the sixth one. Noting the tube which shows complete hæmolysis with the smallest piece of paper we designate that amount of paper as one unit and use twice that amount of paper for the carrying out of the test. This is really a titration of the complement although the varying factor is the amboceptor. For standardization of amboceptor see page 200.

Preparation of Acetone Insoluble Antigen.—Take about 50 grams of finely divided beef, dog, or rabbit heart or liver and triturate in a mortar to a paste. Pour on this paste 500 c.c. of absolute alcohol and keep the mixture in a corked bottle in the 37°C. incubator for five to seven days shaking the emulsion four or five times daily. (We use beef heart and carefully pare away all fat and fibrous tissue and macerate the remaining muscular tissue with absolute or 96% alcohol). Next filter through paper and collect the filtrate in a large shallow dish and hasten evaporation with the aid of a current of air from an electric fan directed upon the surface. It is advisable to cover the dish with a single layer of cheese cloth to prevent access of flies, etc., to the contents. These insects may contaminate the solution with moulds which causes disappearance of the lipoids.

Within twenty-four hours only a sticky residue should remain. This is taken up in about 50 c.c. of ether and the turbid ethereal solution kept over night in the refrigerator in a corked bottle.

In the morning there will be found about 45 c.c. of clear supernatant fluid which is decanted off and allowed to evaporate to about 15 c.c.

Now to this 15 c.c. add about 150 c.c. of acetone and a precipitate will form which collects at the bottom of the measuring cylinder. Now pour off the supernatant acetone and let the sediment stand until it is of a resinous consistence. Now dissolve 0.3 gram in 1 c.c. of ether and then add 9 c.c. of methyl alcohol. This gives the *stock antigen solution* which is crystal clear.

In using this antigen solution for the Emery or Noguchi test we dilute 1 c.c. with 9 c.c. of salt solution. This *opalescent, working, antigen emulsion* should be made up fresh on the day of preparing the tests.

About one-half of these antigens are lacking in power to absorb complement in the presence of syphilitic sera. More rarely they may absorb complement with a nonsyphilitic serum (anticomplementary) or they may have a hæmolytic action. Consequently a new stock antigen should be tested as to its reliability—

1. A mixture of 0.4 c.c. working antigen emulsion, 0.6 c.c. salt solution, and 0.1 c.c. of a 10% suspension of washed red cells when incubated at 37°C. for two hours should not show any hæmolysis.

2. A mixture of 0.4 c.c. working antigen emulsion, 0.6 c.c. salt solution, 0.1 c.c. of a 40% solution of fresh guinea-pig serum, and 2 units of amboceptor and incubated at 37°C. for one hour should show hæmolysis when we now add 0.1 c.c. of a 10% washed red-cell emulsion and the whole then again incubated for two hours at 37°C. (The antigen did not absorb complement in the absence of syphilitic antibodies).

3. A mixture of 0.2 c.c. of a 1 to 10 dilution of working antigen emulsion, 0.8 c.c. of salt solution, 1 drop of syphilitic serum, 0.1 c.c. of a 40% dilution of fresh guinea-pig serum, and 2 units of amboceptor should be incubated at 37°C. for one hour. When we then add 0.1 c.c. of a 10% suspension of washed red cells and again incubate for two hours we should fail to obtain hæmolysis. (The antigen can absorb complement through the intermediation of syphilitic antibodies).

Preparation of Amboceptor Paper.—In order to secure blood from the vein of a man or the heart of the immunized rabbit, the most convenient method is with the use of an Erlenmeyer flask with a rubber stopper having two perforations in the stopper. To one of the projecting pieces of glass tubing a stout hypodermic needle is attached through the medium of about 8 inches of rubber tubing, and the second piece of glass tubing is bent at an angle as it leaves the stopper to provide a suction tube. With a man, constrict the upper arm sufficiently to stop venous return with an Esmarch rubber bandage or a towel. Paint tincture of iodine over a prominent vein at the bend of the elbow. Gentle suction will cause the blood to flow into the needle tube and thence into the flask when the vein is entered.

The blood as it is taken from the arm should be received in about 50 c.c. of normal salt solution containing 1% of sodium citrate. About 28 to 30 c.c. are usually sufficient. Now throw down this red-cell suspension in three or four centrifuge tubes. The resulting sediment should be washed and rewashed with salt solution. Two to three washings with salt solution suffice.

Now take a large healthy rabbit, shave the lower abdomen and paint the surface with tincture of iodine. The easiest way to inject the rabbit is to hold the animal head down and plunge the needle of a large glass hypodermic syringe containing the washed red-cell sediment into the abdominal cavity in the median line. The intestines gravitate downward and by entering the needle below the limits of the bladder we avoid injuring any vital part.

Make the injections at intervals of five days and give increasing amounts at each successive injection. Thus, first injection, 5 c.c.; second injection, 8 c.c.; third

injection, 10 c.c.; fourth injection 12 c.c.; and at the fifth injection give about 15 to 20 c.c. of washed red-cell sediment. It is well to dilute the cell sediment with an equal amount of salt solution. About ten days after the last injection, we take some blood in a Wright's tube from a vein of the ear and dilute the serum to make a 1 to 100 dilution. To 1 c.c. of a ½% emulsion of red cells we add 0.1 c.c. of a 40% dilution of guinea-pig's fresh serum—similar combinations being made in a series of 10 tubes. To each of these tubes we add varying amounts of the 1 to 100 dilution, 0.1 c.c. in the first, 0.2 c.c. in the second, 0.3 c.c. in the third, and so on. If we obtain hæmolysis in the tube containing 0.2 c.c. of 1 to 100 dilution of serum but not in that containing 0.1 c.c. we note that the serum has a titre of about 1 to 500. If the 0.1 c.c. gave hæmolysis, the serum would have a titre of 1 to 1000.

Having ascertained that the hæmolytic serum is sufficiently strong we shave the left side of the thorax of the rabbit and enter the needle of the apparatus similar to that used for taking the blood from a man's vein in one of the intercostal spaces of the left side.

Having introduced the needle, feel for the heart beat and then plunge the needle into the heart. We can withdraw about 30 c.c. of blood without injury to the rabbit. This blood should be received in a clean empty flask and set, over night, in the refrigerator. The following morning pour off the clear serum into a clean Petri dish and saturate, one by one, squares of filter paper with the serum. Allow the filter paper to dry on a piece of unbleached muslin. Noguchi recommends Schleich and Schull's paper No. 597. When thoroughly dry cut strips 5 mm. wide. This makes the amboceptor paper. To standardize, take a series of tubes containing 1 c.c. of a ½% emulsion of red cells and add 0.1 c.c. of 40% dilution of guinea-pig serum for complement. Next cut across the amboceptor paper strip pieces of varying width, as 1 mm., 2 mm., 3 mm., 5 mm., and so on. The narrowest strip which gives hæmolysis in one hour in the incubator or one-half hour in the water bath equals 1 unit. Thus if a piece 5 mm. wide was required to produce hæmolysis, 5 mm. of the paper would have a value of 1 unit.

Coca injects rabbits intravenously with 1 c.c. of well-washed red cells and five days later gives a similar dose intravenously. The blood for the hæmolytic serum is taken from the rabbit five days after the second and last injection. He states that such a method not only gives a high titre but avoids to a great extent agglutination difficulties. It also is more stable as regards holding its original titre.

We have had better success with the following method than with Coca's. Inject intravenously 1 c.c. of 10% red-cell suspension and five days later inject 2½ c.c. of the same strength suspension. Five days later inject 5 c.c. and seven days afterward withdraw from an ear vein, using a Lyon's tube, 4 or 5 c.c. of blood for determining the titre of the serum. If the titre is not satisfactory again inject 5 c.c. of the red cell emulsion.

The Wassermann Test

In the Wassermann reaction the rabbits are injected with sheep red cells which have been washed twice with salt solution by aid of the

centrifuge. About five intraperitoneal injections with, on the average, the quantity of red cells contained in 5 c.c. of sheep blood, given at intervals of five days, gives a strong hæmolytic serum if taken about one week after the last injection.

The standardization of the titre is similar to that for the human hæmolytic amboceptor, except that 5% emulsion of sheep cells with 0.05 c.c. undiluted guinea-pig serum is used instead of the ½% emulsion of human cells and the 0.1 c.c. of 40% guinea-pig serum.

The method is to take in a test-tube 0.2 c.c. inactivated human serum (heated for twenty minutes at 56°C.), 0.1 c.c. fresh serum from guinea-pig for complement, 1 unit antigen and 3 c.c. normal salt solution; then to incubate for one hour at 37°C. (An antigen unit is the amount that will inhibit hæmolysis of 1 c.c. of 5% emulsion of sheep cells when mixed with 0.2 c.c. luetic serum and 0.1 c.c. guinea-pig complement.)

There should likewise be absolutely no inhibition with 0.02 c.c. of serum from a normal individual. The unit is made up to 1 c.c. with salt solution.

Then add 2 units of amboceptor and 1 c.c. 5% emulsion of sheep red cells, shake and incubate for one hour. (The amount of hæmolytic serum that will hæmolyze 1 c.c. of a 5% emulsion of sheep red cells to which 0.05 c.c. guinea-pig serum has been added, in one hour, is an amboceptor unit.)

In our laboratory we use 1 c.c. of a 1 to 10 dilution of the clear stock acetone insoluble antigen of Noguchi as the antigen unit.

In the preparation of the antigen originally recommended by Wassermann, we finely divide the liver of a syphilitic fœtus and extract it for twenty-four hours with salt solution containing 0.5% carbolic acid. Frequent shaking is required. The supernatant fluid is decanted and centrifuged. This turbid fluid is pipetted off and kept in the ice-box in a brown bottle for several days. This yellowish-brown opalescent fluid is the antigen and is standardized so that 1 unit equals the amount which will inhibit hæmolysis of 1-c.c. of 5% emulsion of sheep red cells when mixed with 0.2 c.c. luetic serum, 0.1 c.c. guinea-pig complement and 2 units of amboceptor.

The same technic is employed with the control test-tube except that the antigen unit is not put in.

The Noguchi method has been stated to give a positive reaction with nonsyphilitic sera in about 7% of cases. The Wassermann a negative result in about 9% of syphilitic sera. These figures show the advantage of checking one against the other.

One objection to the Wassermann test is that a majority of human sera show native antisheep amboceptors and in some instances the amount of this constituent may be so great, when added to the 2 units of amboceptor from the serum of the sheep cell immunized rabbit, as to cause hæmolysis with a syphilitic serum. Simon recommends treating the sera to be tested with sheep cells in order to fix these native amboceptors. Centrifugalizing, the sheep cells with the attached amboceptors are thrown down, leaving the clear supernatant serum free of these amboceptors.

There seem to be certain sera when with a clinical history of syphilis we obtain a positive Wassermann with unheated serum and a negative one with inactivated serum. In order to obtain information with the same serum heated and unheated I would recommend, when it is inconvenient to carry out the original Wassermann technic, to employ the Noguchi technic with inactivated serum and the Emery technic with fresh unheated serum. In any case when serum cannot be tested within twenty-four hours it should be inactivated, as unheated serum tends to become anticomplementary.

Emery's Technic for the Wassermann Test

Owing to technical difficulties with the method of making and employing the antigen and amboceptor features of the original Emery test, I have retained the principle of the test but substituted the reagents prepared in exact accordance with Noguchi's directions.

Briefly stated, the principle of Emery's test consists in the employment of fresh human serum for supplying complement and the primary incubating of the hæmolytic system (human red cells and rabbit serum immune to human red cells) at the same time as the incubation of the antigen and serum but in separate tubes. Then in the second period of incubation to add these "sensitized" cells to the serum antigen combination.

In Noguchi's method all reagents are incubated together in the first period with the exception of the amboceptor paper (dried serum of rabbit immune to human red cells), which is not added until the period of incubation for complement binding is completed and the second incubation commenced. Time is saved in the Emery technic, inasmuch as the red cells are already sensitized by the hæmolytic

amboceptor when added to the tubes, and hæmolysis shows itself almost immediately in tubes where the complement has not been absorbed by the antigen through syphilitic antibodies.

Noguchi has called attention to the fact that protein constituents of certain aqueous or alcoholic extracts may have the power to fix complement through certain intermediaries existing in fresh serum which, however, does not obtain for inactivated sera (sera heated to 56°C. for fifteen minutes).

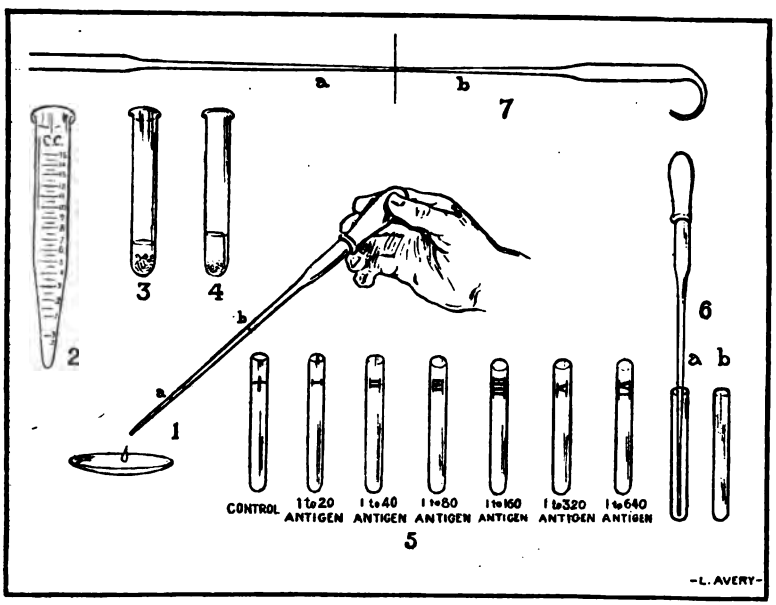


FIG. 51.—1. Capillary pipette being graduated by drawing up 1 and 4 drops from a watch-glass. (a) Blue pencil mark of 1 drop or 1 volume. (b) Mark of volume of 4 drops. 2. Graduated centrifuge tube containing sodium citrate normal salt solution. 3. Tube with 10 amboceptor units in 1 c.c. of salt solution. 4. Mixture of 1 volume 20% emulsion red cells and 4 volumes inactivated amboceptor solution. 5. Small glass tubes for Emery test. 6. Method of transferring from tube to tube. 7. Making a Wright U-tube—the end "*a*" to be used as a capillary pipette.

Pure lipoidal substances as contained in Noguchi's acetone insoluble antigen, however, do not act in this way.

Consequently by using such an antigen we eliminate the objection to employing fresh human serum in the test for syphilitic antibodies.

As giving more uniform hæmolytic results and as being more stable and easier of employment, I have made use of Noguchi's directions for taking up the serum of the rabbits, immunized to human red cells and his method of standardizing this "amboceptor" paper. In practice, I measure off the length of paper correspond-

ing to 10 Noguchi units and dissolve the dried serum in such paper in 1 c.c. of salt solution. This makes a satisfactory and uniform substitute for the sterile immune serum used by Emery.

Method: 1. Take blood from the finger or ear in a large Wright U tube (¼ inch in diameter). Place in 37°C. incubator for fifteen minutes (to increase yield of serum) and then centrifuge.

Of course when the Noguchi test is being made at the same time we use the serum of the blood taken from a vein in the arm.

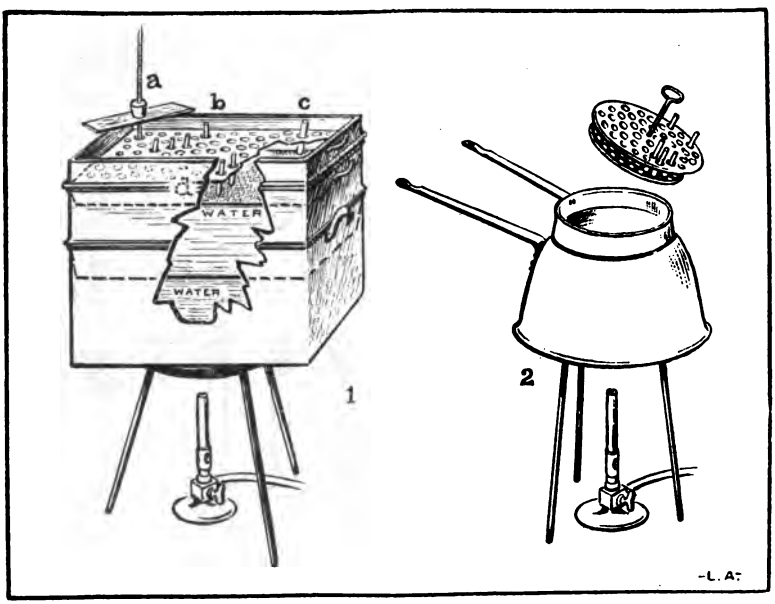


FIG. 52.—1. Copper water bath 12 ×12×8 inches. (a) Thermometer to show 38°C. (b) Tubes containing antigen dilutions. (c) Tube containing *hæmolytic system* incubating along with the antigen tubes. 2. Ordinary rice cooker with copper holder for test-tubes.

2. Graduate a capillary pipette for 1 volume and 4 volumes.

There is the greatest variation in the size of a drop delivered by a capillary pipette, this difference in size not only occurring with varying diameters of the capillary tube but with position of tube, thus a tube with 1 mm. diameter and held horizontally will deliver about 16 drops of serum to the c.c. If held vertically about 32. A fine capillary pipette, such as is used by Noguchi will deliver, when held vertically, 50 drops to the c.c., or 0.02 c.c. to the drop. It is therefore better to standardize with known amounts delivered from a graduated pipette, using $\frac{1}{20}$ c.c. as the unit for 1 volume.

3. Into each of a series of small test-tubes put 4 volumes of normal salt solution. (These tubes are most conveniently made by breaking off 2½- to 3-inch lengths of ¼-inch soft glass tubing and then fusing one end in the flame to make a small test-tube.)

Make a distinguishing mark, *e.g.*, X, on end of tube with blue-wax pencil and use this tube to hold control. Mark the other tubes I, II, III, and so on. When different sera are to be tested they may be distinguished by lines either above or below, or with circles; also marks with red-wax pencil may be used.

4. Make a 1 to 20 dilution of stock antigen solution in salt solution.

To Tube I add 4 volumes of 1 to 20 antigen, thus making 8 volumes of 1 to 40 antigen in Tube I. Mix thoroughly by manipulating bulb of pipette. Then transfer 4 volumes of the 1 to 40 from Tube I to Tube II, and so on through the series. When the dilution in the last tube has been made throw 4 volumes away.

The 4 volumes of dilution of the antigen in the respective tubes will then be: In Tube I, 1 to 40; in Tube II, 1 to 80; in III, 1 to 160; in IV, 1 to 320, and so on.

5. Add 1 volume of serum to be tested to control tube X, and to each of the tubes I, II, III, etc., in succession. (If the serum be added to the antigen tubes before the control tube, antigen might be carried over to the control.)

NOTE.—If the serum has been inactivated restore complement by adding 1 volume of a 40% fresh guinea-pig serum. Also use 2 volumes of this inactivated human serum instead of 1.

6. Incubate at 38°C. for thirty minutes. This allows syphilitic antibody, if present, to bind complement.

7. As soon as the above mixtures have been made and put in the incubator prepare the "hæmolytic system" by adding 1 volume of 20% emulsion of washed human red cells to 4 volumes of solution of amboceptor paper (10 Noguchi units of amboceptor paper dissolved in 1 c.c. of salt solution. Thus of a paper of which 4 mm. was the unit we should cut off about 40 mm., place in test-tube and extract the dried serum with 1 c.c. of salt solution), and place this hæmolytic system in incubator alongside the tubes already there. To obtain the washed red cells allow 4 to 10 drops of blood to drop into a graduated centrifuge tube containing salt solution to which has been added 1% of sodium citrate to prevent coagulation. After shaking, centrifuge. Pour off supernatant fluid, replace with salt solution, again shake and centrifuge—this sediment of red cells is to be diluted with 4 volumes of salt solution (20% emulsion). (Incubation hastens sensitization of the red blood cells. Agglutination of red cells may occur with certain hæmolytic sera. The immunization of the rabbits with small doses intravenously (Coca's method) tends to prevent this interfering factor. However, frequent shaking is usually sufficient to break up the agglutinating masses of red cells).

8. At the expiration of thirty minutes from the commencement of incubation for complement binding, add 1 volume of hæmolytic system to each of the tubes, I, II, III, etc., in the order of antigen dilution.

9. Finally, after washing pipette in salt solution, add 1 volume of hæmolytic system to control in tube X. (If the hæmolytic system should be added to the control tube before the antigen tubes, complement from the control tube might be carried over to the antigen tubes.)

Shake each tube thoroughly. Allow them to incubate for a few minutes. Then examine tubes I, II, III, etc., for hæmolysis. The control should, of course, show hæmolysis. The antigen tubes should show a white, supernatant fluid over the intact red-cell sediment in the tubes with the low dilutions and even in the highest dilutions, where the serum is strongly positive. In a weakly positive serum, inhibition of hæmolysis may only show in the first tube and hæmolysis show in those tubes having higher dilutions of antigen.

It will be noted that the reagents are made in accordance with Noguchi's directions. Even in those cases where fresh guinea-pig serum is employed to replace complement, absent from the inactivated human serum tested, we employ the 40% solution used in Noguchi's technic.

Experiments have shown that one volume of serum contains almost invariably sufficient complement to hæmolyze the red cells present. As a matter of fact in 95% of sera one-half this amount would suffice.

It will be noted that the amboceptor and antigen content of the 1 to 80 tube is proportionate to that used in the Noguchi test.

Noguchi's Technic with Sensitized Cells

There is evidence for believing that the heating of the serum to be examined not only destroys about 75% of its total syphilitic antibody content but may also destroy thermolabile substances which may be of import ance in bringing about a positive reaction. At any rate I have had experience with sera where, with an absolutely clear diagnosis of syphilis followed by response to therapeutic measures, there has been a negative reaction with inactivated serum but a positive one with unheated serum.

For this reason I would advise that the Noguchi method be carried out with inactivated serum and an Emery test also made using unheated serum.

The Emery test is also of value as showing a quantitative relation. At the same time I am convinced that it is more conservative to make a diagnosis of syphilis only with inactivated serum, reserving the utilization of findings with unheated sera for effect of treatment on syphilitics and for such cases as the symptomatology and history of the case would indicate that a positive reaction should be obtained.

No injury is conferred on a patient by a negative Wassermann be cause in the presence of other data treatment will not be withheld.

A modification of the Noguchi technic, along the Emery lines, which saves time and makes readings sharper is to employ sensitized red cells. To do this add to the drop of patient's serum and 40% guinea-pig complement and antigen in tube 1*a*, ½ c.c. of salt solution. Also to tube 1*b*, containing the serum and complement but without antigen add ½ c.c. salt solution. Incubate for absorption of complement. So soon as these tubes are placed in the incubator prepare a mixture of 1% washed red cells, according to the amount to be used in the number of tests to be carried out, and add 2 units of amboceptor paper for each ½ c.c. of this red-cell emulsion. Incubate along with the other tubes. When the period for the primary incubation is completed add to each tube ½ c.c. of the emulsion of sensitized red cells.

Incubate again and readings can usually be made in ten to fifteen minutes and give unusually clear-cut readings.

General Considerations.—Cherry thinks anticomplementary bodies are found during chloroform anæsthesia. If the antigen should also have anticomplementary action the total might give a negative result.

By heating the serum for half an hour at 56°C. (inactivation) the positive results reported to have been obtained in certain cases of cancer, nephritis, scarlet fever, leprosy and tuberculosis may be avoided; the syphilitic antibody alone being more thermostable. The thermostability of serum of inherited syphilis is the highest—that of primary syphilis the least of luetic sera.

McDonagh states that in the primary stage the Wassermann is positive in 40% of cases. In secondary cases 97% give positive results when treatment has not been instituted. In tertiary syphilis about 70% are positive.

In 268 cases at the medical clinic of Johns Hopkins Hospital, Clough failed to obtain a positive reaction in 99 cases which were negative clinically.

In 45 cases of syphilis he obtained 73% of positive results. Excluding cases which had received thorough treatment 82% were positive. Tabes gave 40% and general paresis 100%. In five cases of primary syphilis four gave positive reactions. Kolmer gives 96% positives for untreated active tertiary syphilis with 75 to 80% for latent tertiary syphilis. In untreated congenital syphilis of children over one year of age, 97 to 100%. Comparing the luetin reaction with the Wassermann, Noguchi gives 80% for tertiary and 70% positive for congenital syphilis.

Based upon the observation of Bauer, that human serum contains hæmolytic amboceptors for sheep corpuscles, and of Hecht, that the complement normally present in human serum would suffice without the addition of guinea-pig serum complement, the following method of *Fleming* is easy of application, *but not recommended.*

For the test we use:

1. Alcoholic extract of rabbit's heart, made by washing the recently removed heart with salt solution to remove all blood. Cut into small pieces and grind in a

mortar with sand and for every gram of heart add 5 c.c. of 95% alcohol. Keep the mixture at a temperature of 60°C. for two hours and filter. This is the stock solution. For use dilute it 10 times with normal salt solution.

2. A 5% emulsion of washed sheep red cells, prepared as for the Wassermann test.

3. Suspected and control sera.

With a capillary bulb pipette take up 1 part of serum and 4 parts of the heart antigen, mix on a glass slide, again draw up into the capillary pipette and, leaving a separating air space, next draw up 1 part of 5% emulsion of sheep red cells. Then seal off tip of pipette and incubate at 37°C. for one hour. Now file off tip and mix the red cells with the serum and antigen and again draw up into the capillary pipette and incubate a second time for two hours. Hæmolysis or the reverse is shown in the fluid overlying the cell sediment. Various controls should be made using normal and known syphilitic sera; also with normal salt instead of serum.

The objections to methods using human serum for complement are 1. the great variation in the complement content of different human sera; 2. human complement requires about 10 times as much amboceptor as guinea-pig complement and is less sensitive to fixation, and 3. the statement is made by some workers that while homologous complement and amboceptor may be efficient yet the complement of a serum will not act upon its homologous antigen. This is not true because the complement of human serum invariably hæmolyzes the homologous antigen (human red cells).

The various precipitate tests that have been proposed are unreliable. The precipitate reactions with bile salts give better results than with lecithin, this latter showing positive results in almost one-half of non-syphilitic cases.

Bacterial Complement-fixation Tests

The two bacterial complement-fixation tests which are used as routine diagnostic methods are those for gonorrhœal and glanders infections.

Of course similar tests may be made for typhoid, cholera, etc., but we have more simple and practical methods in the use of agglutination or, in the case of typhoid, blood culture procedures.

The two best-known methods for preparing bacterial antigens are the following:

1. Emulsify the growth on agar or starch agar (for gonococcus) in salt solution, as described under preparation of vaccines. Heat the emulsion at 60°C. for one or two hours and then count the organisms as for vaccines.

For gonococcus test we use an antigen with 4,000,000,000 organisms in 1 c.c. This may be used directly as antigen or it may be shaken up with glass beads for several hours to complete disintegration. The antigen can be preserved by the addition of ¼% trikresol or ½% phenol. For glanders one may use a seventy-two-hour culture in glycerine bouillon, sterilized at 60°C. for two hours and preserved with 0.5% phenol.

2. Besredka and Gay prepare their antigen by precipitating the saline bacterial emulsion, washed-off agar, with an equal amount of absolute alcohol. Then centrifugalize, pipette off supernatant fluid and dry the sediment *in vacuo* over sulphuric acid. The dried sediment is made into a 2% suspension with isotonic salt solution. For use this stock solution is diluted. There are also methods in which the bacterial sediment is frozen with carbon dioxide snow and then triturated with crystals of sodium chloride so as to make an isotonic saline emulsion.

Bacterial sediments can also be dried in calcium chloride desiccators.

In carrying out bacterial complement-fixation tests we use an amount of antigen which will by its antigenic power alone fix complement or, as is often stated, be anticomplementary.

Then use one-half this amount as the antigen content for the test.

The method of Noguchi, as previously described, but using one-half the anticomplementary dose of antigen, is satisfactory after experimenting with the proper amount of inactivated serum of the patient to be examined.

For the *Gonococcus* fixation test it is most important to have antigen prepared from a mixture of several strains of gonococci, preferably 10 or 12.

In our laboratory we have had sharper and more satisfactory readings by employing the Emery technic, placing in the first antigen tube an emulsion containing 4,000,000,000 organisms to the c.c.

It is also satisfactory to have the antigen in tube 1 so concentrated that it will prove anticomplementary. Tube 2 would contain one-half this amount of antigen; tube 3, one-fourth; tube 4, one-eighth; tube 5, one-sixteenth; tube 6, one-thirty-second; tube 7, one-sixty-fourth. Some sera are strong enough in specific gonococcus antibody to bring about complement fixation in tube 8. Along with the test of the patient's serum we should carry out tests with known negative and positive sera.

Determination of Opsonic Power and the Preparation of Vaccines

In that which has been considered in the previous pages only the theories of Ehrlich have been brought out. In order to understand the problems involved in the study of opsonins the phagocytic theory of

immunity brought forward by Metchnikoff must be studied. Ehrlich's views would seem to hold with diseases where there is an increase in bacteriolytic or antitoxic power of the serum while in such diseases, as are caused by pathogenic cocci, the phagocytic element is operative as there is an absence of bacteriolytic power in the serum of the person with the infection.

There are two kinds of phagocytes, the microphages (represented by the polymorphonuclears) which on phagolysis or disintegration give off microcytase, a bactericidal substance. *Cytase* is the same as complement or alexine.

The microphages are chiefly bactericidal while the macrophages, represented by the large mononuclears of the blood and fixed connective-tissue cells, exert their action on protozoa or animal cells.

Phagocytes may either act by ingesting bacteria and destroying them intracellularly or they may as a result of phagolysis bring about bacteriolysis extracellularly. According to Metchnikoff the intracellular bacteriolysis explains why an individual may possess immunity and yet his serum fail to show any bacteriolytic power.

The control of vaccine treatment by taking opsonic indices from time to time does not seem to have met with much favor in this country—the sources of error being as great if not greater than ordinary variations in the opsonic index during the negative and positive phases.

Method of Wright for Obtaining Opsonic Index

While other observers had previously noted the presence of substances in immune sera which so acted on the bacteria that phagocytosis was made possible, yet it was to Wright and Douglas, in 1903, that the existence of this factor in phagocytosis was brought forward and the estimation of such substances made practicable.

To this substance the name *opsonin* was given—the Greek word from which it is derived indicating preparation of the food—that is, the opsonin so alters or sensitizes the bacteria that they can be engulfed or phagocytized by the polymorphonuclear leukocytes (the microphages of Metchnikoff). About the same time Neufeld and Rimpau noted the presence of a substance in immune sera which so acted on bacteria as to prepare them for phagocytosis. Their designation "bacteriotropic substance" is practically synonymous with opsonin.

In 1902 Leishman introduced the method of determining the "phagocytic index." By taking 1 part of blood and 1 part of an emulsion of the bacteria in question and keeping the mixture in a moist chamber at body temperature for a standard time,

as fifteen to thirty minutes, and then spreading the blood-bacteria mixture and staining the film with Leishman or Wright's stain he counted the number of bacteria in a certain number of polymorphonuclears, and by dividing obtained the average number per leukocyte of bacteria phagocytized.

The Wright technic for determining the phagocytic average, and from this the opsonic index, is as follows:

Blood is taken from the patient and at the same time from a normal individual, or preferably the blood of several normal individuals is pooled. This blood is best collected in a Wright's tube, although it may be received in a small test-tube. After coagulation and separation of the serum, the serum is ready for use.

The next step is to prepare the leukocyte emulsion. For this we fill a centrifuge tube with normal salt solution, to which has been added 1% sodium citrate —the latter to prevent coagulation. Then having pricked a finger congested by a constricting rubber band, from 15 to 20 drops of blood are added to the citrated salt solution, and the mixture thoroughly shaken. After centrifugalization for about five minutes the red corpuscles will be thrown to the bottom of the tube with the leukocytes forming a superimposed layer. In order to free the leukocytes entirely from serum admixture, the supernatant citrated salt solution is pipetted off, and a fresh tubeful of salt solution is added to the blood-cell sediment. Again shaking, we centrifuge, obtaining for a second time a sediment of blood cells with the leukocytes in the superimposed layer. In some laboratories the washing in salt solution is again repeated, but for all practical purposes two washings as described above suffice.

The superimposed layer of white cells may now be pipetted off from the heavier red cells (of course, containing a large admixture of red cells) to be used as a leukocyte cream—or by slanting the centrifuge tube we can pipette off the proportion of the leukocyte mixture needed from the bottom, sides or top of the slanted layer of blood cells.

Having prepared our leukocyte emulsion, and the serum from the normal individual as well as that from the patient, it only remains to prepare our bacterial emulsion. For bacteria in general, with the exception of tubercle bacilli, we simply take up a small loopful of a young agar culture (eighteen hours or less), and emulsify it uniformly with salt solution, added by degrees until the suspension amounts to ½ to 1 c.c., and giving a faint turbidity. To thoroughly distribute and especially to break up clumps repeated suction and ejection with a capillary pipette provided with a rubber nipple is satisfactory.

The presence of clumps in a bacterial emulsion invalidates the estimation of phagocytosis, for the reason that a leukocyte will take up a clump of twenty or more bacilli as readily as one separate organism.

Having at hand (1) the suspension of leukocytes, (2) the bacterial emulsion, and (3) the sera of the patient and the normal individual, we are ready to proceed with the test.

Using a capillary bulb pipette with a pencil mark to indicate 1 volume we draw up to the mark (1) the leukocyte cream. Then wiping off the tip of the pipette we draw up this volume of leukocyte emulsion about ½ inch to make an air break

between this and (2) 1 volume of the bacillary emulsion. Again making an air space we draw up (3) the serum of the normal individual. This gives three columns in the capillary tube with intervening breaks of air. We next eject the three constituents into a watch-glass and thoroughly mix them by alternate suction and ejection with the tube and nipple. When mixed we draw the mixture up into the same capillary tube, seal off the capillary end in the flame and put in an incubator for exactly fifteen minutes.

We next repeat the process identically except that the patient's serum is used instead of that of the normal individual.

These tubes having been kept at the same temperature for the same length of time are then taken out, the contents blown into a watch-glass, mixed thoroughly a second time, and then a smear is made—a drop of the mixture being deposited on a very clean slide and the smear made by a second narrower slide (by cutting off the corner of the slide) which is drawn along in a zigzag way. The smears are then stained (Leishman's or Wright's blood stain or Ziehl-Neelson's for tubercle bacilli) and the number of the bacteria in from 50 to 100 leukocytes counted. This number divided by the number of cells gives the phagocytic average.

The phagocytic average of the patient's tube divided by that of the normal individual's tube gives the opsonic index. Thus, in counting 100 cells we find 500 phagocytized cocci in the patient's tube, giving an average of 5, and in the normal individual's blood we get 1000, an average of 10. Then the opsonic index would be $5 \div 10$, or 0.5.

Preparation of Vaccines

It has been found satisfactory to make use of stock vaccines in gonorrhœal and tuberculous affections. In treatment of tuberculosis Wright prefers Koch's T. R. or Neu Tuberculin in doses of from $\frac{1}{5000}$ to $\frac{1}{800}$ mg. Some prefer Koch's more recent bazillen emulsion. In case of other infections, however, and preferably with gonorrhœal infections, the causative organism should be isolated from pus, sputum, urine, blood, or other material from patient (*autogenous vaccine*).

In the making of vaccines all media and apparatus should be sterilized with scrupulous care to avoid the danger of tetanus infection. Having isolated the organism, it is inoculated upon one or more agar slants, and after a growth of from five to seven hours with streptococci and pneumococci, or with eighteen hours for staphylococci and colon, the growth on these inoculated slants is taken up with salt solution, thoroughly shaken up in the diluting solution and standardized. (Esmarch roll of nutrient agar may be inoculated for growing cultures for vaccines.)

The most practical way is to gently rub off the growth on the agar in about 1 or 2 c.c. of salt solution with a platinum loop or sterile cotton swab. Then pour the bacterial emulsion into a sterile test-tube and repeat the process with three to five agar slants, until we have from 6 to 10 c.c. of the emulsion in the sterile test-tube. By heating to melting-point in the flame a piece of glass tubing and attaching it to

the rim of the test-tube (also melted), we have a handle with which to draw out the test-tube when heated about 1 inch from the mouth in a blowpipe flame. Drawing this out, we let it cool, and then filing the constricted portion we break it off and seal it in the flame. By shaking up and down vigorously for five to fifteen minutes, or preferably in a mechanical shaker the bacteria are distributed evenly in the salt solution. A piece of platinum wire, twisted into corkscrew shape, and fused in the drawn-out end of the containing test-tube helps in breaking up the bacterial emulsion and is a great aid in the preparation of streptococci or diphtheroid vaccines.

The sealed test-tube is then placed in a water bath at 60°C. and heated at this temperature for one hour. Again shake. The constricted sealed end is again filed off and a few drops shaken out in a watch-glass for standardization, and at the same time a few drops are deposited on an agar slant as a test for sterility. (Incubation for twenty-four to forty-eight hours should not show growth.)

Wright found that by taking a definite quantity of blood and a similar quantity of bacterial emulsion, mixing the blood and bacterial emulsion, then making a smear and staining, it was possible to determine the ratio of bacteria to red cells, and from this the number of bacteria per cubic centimeter could be determined. For example, if we find three bacteria to each red cell we should have 15,000,000 bacteria to 1 c.mm. (There being 5,000,000 red cells to the cubic millimeter.) As 1 c.c. is 1000 times greater than 1 c.mm., there would be 15,000,000,000 bacteria in each c.c. of such an emulsion, or vaccine, as it is termed.

The standardization made with a hæmacytometer is best done by drawing up the vaccine to 0.5 with either the red or white pipette, according to concentration, and then sucking up one-twentieth of 1% dahlia in 1% formalin to 11 or 101. Allow the bacteria to settle on the shelf for ten minutes before counting. Count as in making a red count.

The use of a piece of amber glass in front of an incandescent light enables one to pick up the bacteria more satisfactorily as well as to differentiate bacteria from débris. A counting chamber with a depth of $\frac{1}{50}$ mm. is to be preferred to the ordinary $\frac{1}{10}$ mm. chamber as one can begin the count after about five minutes time for settling.

A satisfactory diluting fluid is that recommended by Callison. It is: Hydrochloric acid 2 c.c., Bichloride of mercury (1 to 500 aq. sol.) 100 c.c., and sufficient 1% aqueous solution of acid fuchsin to color the diluting mixture a deep cherry red. The diluting fluid should then be filtered. The bichloride forms an albuminate on the surface of the bacteria which promotes rapid sedimentation and the fuchsin stains the bacteria.

Having determined the strength of the stock vaccine, we should prepare a dilute vaccine for injection.

This is most conveniently carried out by filling vials with 50 c.c. of salt solution, plugging with cotton, then sterilizing in the autoclave. A sterile rubber cap is now drawn over the mouth of the vial. Sterility is insured by plunging the rubber cap and neck in boiling water. If the stock vaccine showed 5,000,000,000 bacteria per c.c. and we desired to have a vaccine containing 200,000,000 bacteria per c.c., it would be necessary to draw out 2 c.c. of the salt solution by means of a sterile syringe needle inserted through the rubber cap and replace it with 2 c.c. of the bacterial emulsion. Example: In introducing 2 c.c. of a vaccine containing 5,000,000,000 bacteria per c.c., we throw in 10,000,000,000 bacteria in a volume equal to 50 c.c. Then each c.c. of the 50 c.c. in the bottle would contain 10,000,000,000 divided by 50 or 200,000,000 in each c.c. If we only want a vaccine containing 100,000,000 per c.c. we should only add 1 c.c. We now add ¼% of trikresol to the vaccine in order to insure sterility. (Introduced with syringe, inserting needle through rubber cap.) The syringe is best sterilized by drawing up vaseline or olive oil heated to 150°C., and the neck and rubber cap of the bottle in boiling water. We now draw up the desired dose of bacteria. If glass syringes are used, simply boiling in water suffices. One can purchase ampoules which are sterilized and filled with a standardized emulsion. They are sealed in the flame and labelled with the bacterial content per c.c.

The ordinary doses are: For gonococci, streptococci, pneumococci, and colon vaccines, 5,000,000 to 50,000,000. For staphylococci 200,000,000 to 1,000,000,000.

Wilson gives the following minimum and maximum doses expressed in millions:

Streptococcus, 6 and 68.
Gonococcus, 45 and 900.
Meningococcus, 300 and 900.
M. melitensis, 700 and 1400.
B. coli, 16 and 240.
B. typhosus (treatment) 100 and 250.
B. typhosus (prophylaxis) 500 and 1000.
B. pyocyaneus, 34 and 1000.
B. pneumoniæ, 44.
Staphylococci, 150 and 900.
B. tuberculosis $\frac{1}{20000}$ to $\frac{1}{200}$ mg.

Sensitized Vaccines.—These are prepared by treating the bacteria with the specific serum and cause less reaction than ordinary vaccines. To prepare them the bacterial growth is treated with its antiserum for three hours, thrown down in the centrifuge and the supernatant serum removed. After washing in salt solution they are emulsified in salt solution and killed at a temperature of 56°C. for one hour. Besredka has used living sensitized bacteria in typhoid.

The question of the best method of preparing vaccines for prophylactic use is still unsettled. The greatest difficulty has been experienced in making vaccines of the Shiga bacillus on account of the great toxicity of such preparations.

Thompson has recently carried out some very important experiments at the Lister Institute.

He worked with vaccines heated to 56°C. for one hour using ordinary methods as well as organisms sensitized by treatment with specific serum. In another series of vaccines he sterilized ordinary cultures as well as sensitized ones with 0.5% carbolic acid in normal saline. He found that sterilization by heat not only destroyed much of the immunizing power of the vaccines, but that such vaccines, whether of ordinary bacterial emulsions or of sensitized organisms, showed great toxicity upon their being injected and the heated sensitized ones were somewhat more toxic than the nonsensitized organisms. Dean has used "eusol" for Shiga vaccines.

On the whole it would seem that sterilization with ½% carbolic or ¼% trikresol using ordinary bacterial emulsions, is better than other methods.

Of course living organisms subjected to their specific serum have been recommended in the case of typhoid but such methods are certainly not devoid of danger and are not to be recommended for the present.

General Considerations as to Vaccine Therapy.—While there is indisputable evidence as to the value of bacterial vaccines in prophylaxis, as shown in typhoid prophylaxis, yet with the use of these vaccines as therapeutic measures there is considerable evidence to minimize their value, as shown by Leake in a recent article. Whittington divided 230 cases of typhoid into two groups, one receiving vaccines, the other treated in the ordinary way. His results were as follows:

	Mortality Per Cent.	Average Days Fever	Relapsed Cases Per Cent.	Cases with Complications or Sequelæ Per Cent.
Vaccine-treated cases.....	25	29.2	10.4	49.5
Controls.....	21	26.1	7.8	46.0

Park tested the value of pertussis vaccines in the treatment of a large number of cases of whooping cough, selecting alternate cases for the vaccine treatment. It was found that the specific treatment showed no superiority over non-specific ones.

Intravenous injections of bacterial proteids, usually with the use of typhoid vaccines, have been much advocated for chronic joint affections. It has been found very dangerous to exceed 0.3 c.c. of the standard typhoid vaccine and we have noted one fatal result, with anaphylactic death, at the time of the second intravenous injection of such a vaccine.

There is no question but that in many cases of furunculosis we obtain brilliant results with autogenous staphylococcus vaccines.

ANAPHYLAXIS

This is a term which indicates the opposite of prophylaxis. It was noted that after a period of incubation of at least ten days a second

injection of horse serum produced symptoms of respiratory embarrassment, convulsions and, at times, death. The primary injection had during the period of incubation sensitized the cells to this particular proteid. Extremely small amounts of serum will sensitize (0.0001 c.c.). For anaphylaxis production much larger amounts are required; from 0.01 to 0.3 c.c. when given intravenously or intracardially and 1 or 2 c.c. when given subcutaneously.

This phenomenon of sensitization in the case of rabbits bears the name of Arthus, and as applied to guinea-pigs sensitized with diphtheria antitoxin sera the name Theobald Smith, and it is stated by Muir and Ritchie that active research as to anaphylaxis may be said to date from the discovery of the *phenomenon of Theobald Smith.*

Rosenau and Anderson working with guinea-pigs showed that small doses were efficient for sensitization, that the condition was transmissible from mother to offspring and that a second animal could be sensitized by being injected with the serum of a sensitized animal.

This group of symptoms, the so-called *anaphylactic shock*, which is apt to set in within a few minutes after the second injection, is often preceded by restlessness and great excitement and together with the dyspnœic manifestations such as coughing and rapid breathing, there is cardiac weakness and great fall of blood pressure. The more serious symptoms as convulsions and at times death in from a few minutes to an hour are more apt to appear after intracerebral injections than after intraperitoneal. Subcutaneous injections are least apt to produce anaphylactic symptoms. Our attention to this phenomenon commenced with the study of "serum sickness" or "serum disease." In this an erythematous rash or urticaria associated with more or less œdema comes on after eight to twelve days from the time of the first and only injection of horse serum. It is supposed to be due to the fact that some of the serum originally injected remains unchanged in the tissues so that when the sensitization takes place there is present and at hand the same foreign proteid to bring about anaphylactic symptoms.

Immunization against anaphylaxis is possible by repeating injection of the sensitizing serum or proteid during the period of incubation. When a sensitized animal recovers from the second injection it is afterward immune to anaphylaxis. This is termed antianaphylaxis.

It is important to note that this hypersusceptibility appears to be very rarely of importance in the matter of the administration of a second injection of diphtheria antitoxin after the period of anaphylactic incubation.

As a rule the death or untoward effects of the injection of serum are in cases of status lymphaticus. Cases in man do occur, however, but

with extreme infrequency, in which within a few minutes after the only injection of serum the patient becomes restless, shows symptoms of cardiac and respiratory embarrassment and may be dead in a very short time.

According to Rosenau and Anderson individuals who have asthmatic tendencies as well as those who have had serum injections ten to twelve days or longer prior to the second injection should be considered as possible subjects for anaphylactic shock.

Vaughan recommends that when this is to be feared one should only give about 0.1 c.c. of the serum and after an interval of two hours, provided no untoward symptoms set in, to give the full amount of the injection. Besredka advises heating the serum to 56°C. as a guard against anaphylactic shock.

Where large doses of serum are to be given intravenously, as in type I pneumonias, a skin test should be made to see whether the patient is highly sensitive to horse serum. For this inject intradermally about 0.2 c.c. of a 1 to 10 saline diluted horse serum. If sensitiveness exists a genuine urticarial wheal will develop in about five minutes to begin to fade away after an hour or so.

Even where patients do not show sensitization it is well to give a subcutaneous injection of 1 c.c. before giving the serum intravenously.

Where a patient shows marked sensitization, give very small doses subcutaneously every half hour, commencing with $\frac{1}{50}$ c.c. and gradually going up to 1 c.c. It may be well to follow up the subcutaneous desensitization with small doses, intravenously, at half-hour intervals.

Allergy.—The condition of hypersusceptibility or anaphylaxis is at times termed allergy. Thus in a person who has been successfully vaccinated a reaction shows at the site of inoculation within twenty-four hours which does not appear in the nonimmune person for a period two or three times as long. The diagnostic tests with tuberculin and luetin are hence often referred to as allergic reactions.

Toxic Protein Split Products.—It may here be stated that some investigators are of the opinion that our views not only as to immunity but as to the essential nature of infectious diseases may be later on found to rest in production of anaphylaxis. According to Vaughan and others the parenteral introduction (hypodermic as opposed to alimentary tract or enteral introduction) of foreign proteids excites the formation of specific ferments in the cells and fluids of the animal injected. After the ferment is formed a second injection of the same

proteid activates the ferment which splits up the proteid into a poisonous and nonpoisonous portion, the former causing the symptoms of anaphylaxis or disease in the case of the poisonous split proteid of bacterial pathogens.

The name *anaphylactine* has been applied to the sensitizing substance produced during the period of incubation, and *anaphylatoxin* to the poisonous part of the split proteid.

It has been proposed to employ this phenomenon as a diagnostic measure. By taking the serum of a tuberculous patient, which would contain the sensitizing substance, and injecting it into the peritoneal cavity of a rabbit, the animal would be sensitized and an injection of tuberculin a few hours later would bring about the phenomena of anaphylaxis in the rabbit.

This passive anaphylaxis, as it is termed, usually requires approximately twenty-four hours for sensitization. This passive anaphylactic sensitization seems to disappear in two weeks. It has been advised to passively sensitize guinea-pigs with the serum of the person about to be injected and then twenty-four hours after inject the guinea-pigs with the curative serum. If untoward results occur in the guinea-pigs the patient should not receive the injection.

Recently Hagemann has found the following technic valuable in the diagnosis of surgical tuberculosis. Guinea-pigs are inoculated intraperitoneally with tuberculosis cultures and by the end of the second week such pigs are sensitized. The suspected material, as serous effusion, is injected intracutaneously and within twenty-four to forty-eight hours a distinct swelling of the skin with a bluish-red center, which is surrounded by a porcelain white ring and outside of this a zone of inflammation, shows a positive test.

The Abderhalden Reaction

According to Abderhalden, specific ferments of a protective nature (abwehrfermente) appear in the circulation following parenteral injection of various materials and are different from ordinary antibodies.

The substance which causes the production of these specific ferments is called the *substratum* instead of *antigen*, the usual designation of antibody stimulating substances. The principle of the test is that when serum containing such specific ferments is placed in contact, *in vitro*, with the substratum the latter is digested with the production of soluble products, which can pass through a dialyzer and be recognized in the dialysate. The special dialyzers are called thimbles and those prepared by Schleicher and Schull are recommended.

About 0.5 gram of the substratum is placed in a thimble, which has been introduced into a clean Erlenmeyer flask and covered with 1.5 c.c. of the patient's serum.

The thimble is then withdrawn, its open end closed by forceps, then thoroughly washed with distilled water and again introduced into a clean Erlenmeyer flask containing 20 c.c. of sterile distilled water. Toluol is then introduced into the flask so as to cover the water around the thimble. These flasks are then put in the incubator for eighteen hours and the dialysate tested for protein by the ninhydrin test. For this one uses 0.2 c.c. of a 1% solution of ninhydrin in water and adds 10 c.c. of dialysate and the mixture brought to a boil in a test-tube. A positive reaction is a violet blue.

The biuret reaction may also be used.

The difficulties in the way of handling the thimbles and preparing the substratum are so great that Abderhalden questions the competency of even experienced serologists for the carrying out of the test.

The main objection to the test, however, is that it is affected quantitatively so that using varying amounts of reagents one may obtain contradictory results; thus it is possible to obtain ninhydrin reactions with the serum of a male animal acting upon a placental substratum.

While the test is best known in connection with the recognition of pregnancy it has also been employed for the diagnosis of malignant tumors, etc.

In taking serum for the test it is necessary to withdraw the blood as long after a meal as possible, preferably in the morning before breakfast. The blood is taken into paraffin-coated centrifuge tubes and most thoroughly centrifuged, so that it is absolutely free from red cells. It is pipetted off and kept on ice.

For the substratum the placenta should be obtained as soon after delivery as possible or the malignant tumors as soon after operation as is feasible. All connective tissue, blood, etc., should be removed from the tissue from which the substratum is to be prepared.

The tissue is then cut up finely, put in a deep jar and washed several times with distilled water. It is then boiled in a great excess of distilled water—100 times as much—and boiling continued about thirty minutes. This water is decanted and the tissue added to fresh boiling water, repeating the process five or six times until when placed in a dialyzing thimble no ninhydrin reacting soluble proteins can be recognized in the dialysate by the blue color.

The sterile tissue is now placed in a jar, covered with distilled water and on top of this toluol is deposited to maintain the sterility. To estimate the proper amount of substratum to use in a test one must determine this using normal as well as specific sera, recognizing the quantitative error of the test. The amount usually called for is 0.5 gram.

Bronfenbrenner is of the opinion and has supported it by much experimental work that it is not digestion of the substratum which

takes place but autodigestion of the specific serum when placed in contact with its substratum, the products of such autodigestion being of the nature of anaphylatoxins.

Anaphylatoxin Production Test. Bronfenbrenner's Modification of Abderhalden's Test.—Bronfenbrenner found that if he treated the serum of an animal with a particular substratum and then injected the autodigested serum intradermically he obtained a skin reaction. This reaction, however, only obtained for homologous animals, those of a different species not reacting satisfactorily.

He now reports satisfactory results with the following technic: About 2 c.c. of the patient's serum is injected intraperitoneally into a guinea-pig, thus passively transferring to the guinea-pig the specific substances of the human patient's serum. The next day the guinea-pig is bled, its serum collected and placed on ice with a suitable amount of substratum (placenta, bazillen emulsion, tumor tissue, etc.).

Eighteen hours later the serum is separated from the substratum by centrifugalization, pipetted off and placed in the incubator for fifteen hours. At this time inject 0.05 c.c. of the autodigested serum, possibly containing anaphylatoxin, into the shaven skin of a normal guinea-pig.

From twelve to twenty-four hours later a distinct skin reaction occurs at the site of injection, provided the serum of the patient contained antibodies.

Instead of the skin reaction one may inject 0.5 c.c. of such autodigested serum into the heart or vein of a normal guinea-pig and death will result of anaphylactic shock.

If the patient's serum were negative the injection of as much as 5 c.c. would not cause symptoms.

PART II
STUDY OF THE BLOOD

CHAPTER XIII

MICROMETRY AND BLOOD PREPARATIONS

Micrometry

In the examination of blood and fæces preparations, especially when the identification of animal parasites is in question, there is nothing that assists more than a knowledge of the measurements of the object studied. The making of such measurements microscopically is termed micrometry.

Micrometry is also indispensable in bacteriology and cytodiagnosis as well as in animal parasitology.

The most practical way of making these measurements is with an ocular micrometer. These can be bought separately, or a glass disc (disc micrometer) with lines ruled on it can be dropped into the ocular to rest on the diaphragm inside the ocular. The ruled surface of this glass diaphragm should be placed downward. As was stated in connection with the microscope, the image of the object is formed at the level of the diaphragm rim inside the ocular, consequently the lines of the image cut those of the lines ruled on the glass in the ocular. Once having standardized the value of the spaces of the ocular micrometer for each different objective, all that is necessary subsequently in measuring is to count the number of lines or spaces which the image of the object fills and then, knowing the value of each space for that objective, to multiply the number of spaces by the value of a single space.

The Micron.—The unit in micrometry is the micron. This is usually written μ and is the $\frac{1}{1000}$ part of a millimeter. There are 1000 microns in a millimeter.

The Stage Micrometer.—It is possibly more accurate to use a regular stage micrometer scale with ruled lines separated from one another by $\frac{1}{10}$ mm. (100μ). Some of these $\frac{1}{10}$ mm. spaces are again ruled with 10 lines giving spaces which are only $\frac{1}{100}$ mm. (10μ) apart. As one may not have a micrometer scale

similar to that just described, but always has a hæmacytometer, it is better to use such a scale for standardizing the ocular micrometer. In any system of ruling of the hæmacytometer, whether it be the Thoma-Zeiss, Türck or Neubauer one, we have in the central ruling of crossed lines small squares which are used for counting red cells. These are in groups of 16 and each one is $\frac{1}{20}$ mm. or 50μ square.

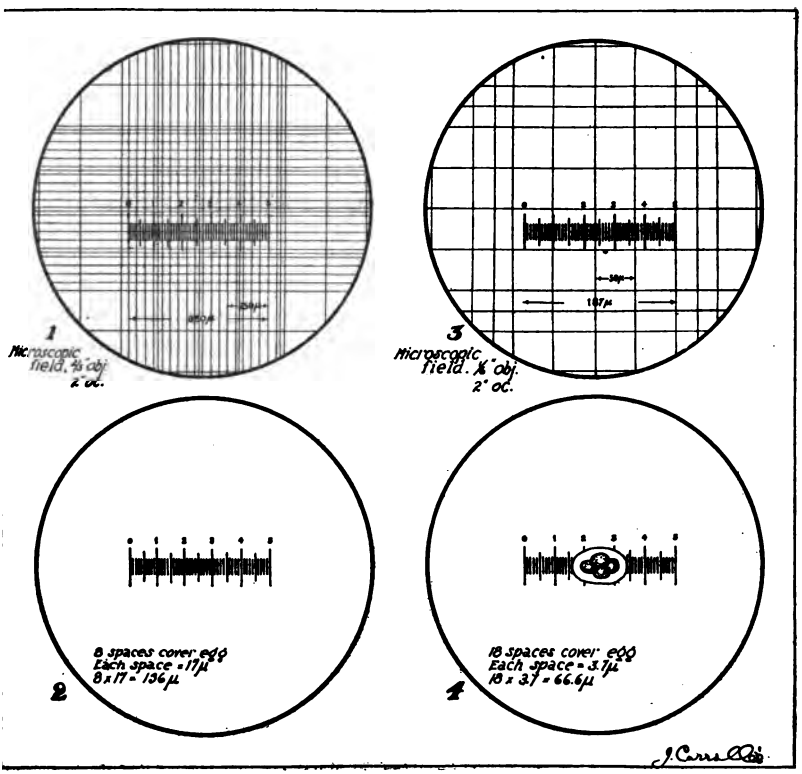


FIG. 53.—1. Fifty lines of ocular micrometer covering 17 small square spaces (50μ each) of hæmocytometer. Each ocular micrometer space = 17μ. 2. Schistosome egg fills 8 spaces of ocular micrometer. Egg is 136μ long. 3. Fifty lines of ocular micrometer covering 3.75 small square spaces. Each space = 3.7μ. 4. Hookworm egg fills 18 ocular micrometer spaces. Egg is 66.6μ long.

Ocular Micrometer.—The ocular micrometer is usually ruled with 50 or 100 lines or spaces, separated by longer lines into groups of 5 and 10.

Having focussed the ruling of the hæmacytometer we note the number of small squares covered by the 50 or 100 ruled lines of the ocular micrometer and multiply

the number of squares so overlaid by 50, which gives the micron value of the entire ocular micrometer ruled space. To obtain the value of each space divide by 50 or 100 according to the number of lines of the ocular micrometer. Having standardized the ocular micrometer for each objective we make a note of such values and need not again repeat the standardization. To measure the egg of an intestinal parasite in fæces we simply focus the egg and note the number of spaces covering it and multiply this number of spaces by the value in microns of the space for the objective used. I always keep the micrometer disc in the 2 inch ocular so that it is always ready for instant use. As a rule clinical microscopists use the 1 inch ocular as a routine one so that the ocular chiefly used is free of any interfering lines. There is a very slight interference with the view of the microscopic field, when using an ocular micrometer.

The tube length which is used at the time of standardizing must always be adhered to in subsequent measurements.

Filar Micrometer.—The most accurate instrument for measuring is the filar micrometer. These are expensive. Measurements can also be made with the camera lucida, but it takes considerable time to make the adjustments necessary, so that it is not convenient. With an ocular micrometer one can make measurements of blood-cells, amœbæ, etc., in a few seconds—it only being necessary to slip in the ocular micrometer.

Rule for determining the magnifying power of microscopic lenses: Measure the diameter of the lens of the objective in inches—the approximate equivalent focal distance is about twice the diameter. Dividing 10 by the equivalent focal distance gives the magnifying power of the lens. This should be multiplied by the number of times the ocular magnifies. Example: The diameter of the lens of the objective was found to measure ½ inch, the focal distance would then be about 1 inch. Dividing 10 by 1 we have 10 as the magnifying power of the lens of the objective. If we were using a No. 4 ocular, the magnifying power would be approximately forty.

Blood Preparations

To obtain blood, except for blood cultures, use either a platino-iridium hypodermic needle which can be sterilized in the flame, a small lancet, or a Hagedorn surgical needle with cutting edge. The blunt end of the surgical needle may be inserted in the cork of a small homœopathic vial. By filling the vial with alcohol we have the needle kept under aseptic condition and just before using we can burn off the film of alcohol coating the needle in the flame.

Puncturing the Skin.—When using such surgical needles it is a good plan to sharpen the cutting edge on a fine-grained whetstone. Afterward the needle should be sterilized by boiling. Sterilization of a needle in the flame blunts the cutting edge. A steel pen with one nib broken off or the glass needle of Wright may also be

used. To make a glass needle, pull straight apart a piece of capillary tubing in a very small flame. Tap the fine point to break off the very delicate extremity. Scarcely any pain attends the use of such a needle. In puncturing either the tip of the finger or lobe of the ear a quick piano-touch-like stroke should be used. The ear is preferable, as it is less sensitive and there is less danger of infection. Before puncturing, the skin should be cleaned with 70% alcohol and allowed to dry. It is advisable to sterilize the needle before using it.

The first drop of blood which exudes should be taken up on the paper of the Tallquist hæmoglobinometer, using subsequent ones for the blood pipettes and smears. If it is necessary to make a complete blood examination, it is rather difficult to draw up the blood in the pipettes, dilute it, and then get material for fresh blood preparations and films without undue squeezing, which is to be avoided. Of course, fresh punctures can be made. Ordinarily, complete blood examinations are not called for. It is only a white count or a differential count or an examination for malaria that is required.

Routine Blood Examinations.—As a practical point it is very rare that a red count is indicated. There is one point not sufficiently recognized by physicians and that is that a routine blood examination is not apt to be as carefully conducted as one calling for a specific feature. Without disparaging the necessity of routine examinations of urine as well as blood it is a fact that the internist who knows what he wants gets better results from the laboratory man.

Hæmoglobin Estimation

Miescher Hæmoglobinometer.—The most accurate instrument for this purpose is the Miescher modification of the v. Fleischl hæmoglobinometer.

The magenta-stained glass wedge for comparison with the diluted blood is similar in each instrument, but by the use of a diluting pipette accurate dilutions are possible in the Miescher. There are two cells provided—one 12 mm. high, the other 15 mm. the idea of this being to enable one to make separate comparisons and to select the central part of the glass-wedge scale, where comparison is more accurate than at the ends. As these cells contain columns of diluted blood proportionately as 5 to 4, we should have similar readings when we multiply the reading on the scale with the 15 mm. cell by $\frac{4}{5}$.

The mixing pipette is graduated with the marks $\frac{1}{2}$, $\frac{2}{3}$ and $\frac{1}{1}$—the first giving a dilution of 1 to 400 (when the diluent, a 0.1% soda solution, is drawn up to the mark above the bulb) the second of 1 to 300 and the last of 1 to 200.

Artificial light preferably from a candle is necessary. There is a table accompanying each instrument which shows the value for that particular instrument in

milligrams per liter of hæmoglobin for any reading obtained on the scale. The normal amount of hæmoglobin in the blood is usually given as 13 to 14 grams per 100 c.c. of blood. For the first two weeks after birth the amount is much higher, 16 to 20 grams per 100 c.c. After this time it begins to drop so that a child from five or six months to twelve or fifteen years old only has about 11 grams in 100 c.c. of blood.

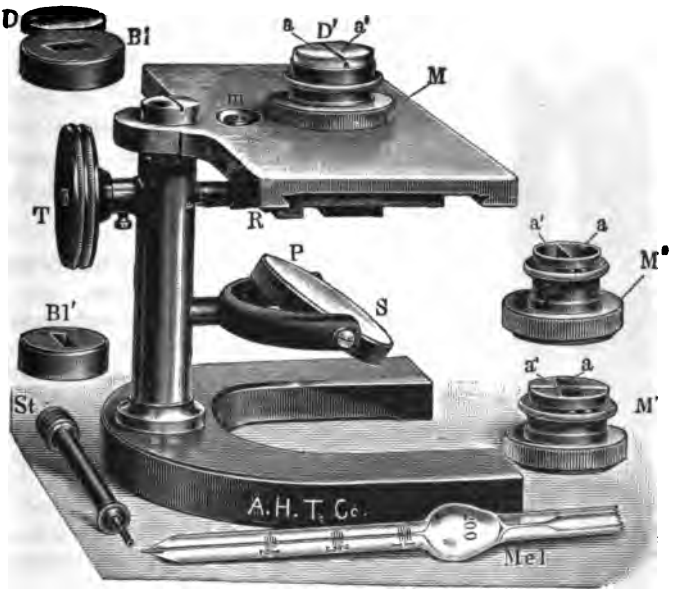


FIG. 54.—Von Fleischl's Hæmoglobinometer (Miescher's Modification).—Description.—Milled wheel at left (T) moves a tinted glass wedge (R) under the fixed metal stage surmounted by double chamber reservoir which receives light from the calcium sulphate reflecting disc (PS) below. Half of the same chamber is filled with the diluted blood contained in the measuring capillary pipette (Mel). The other contains only plain water but receives its light from the colored wedge. By moving the wedge back and forth the colors are matched and the percentage reading is shown on a scale visible through the opening (M) just in front of the supporting upright. The blood is obtained in exactly the same manner as for a blood count, the diluent being calcium carbonate solution (0.1 per cent.) and the tube permitting the observer to use dilutions of 1 : 200, 1 : 300 or 1 : 400 according to the height to which the blood column is allowed to rise (marks $\frac{1}{1}$, $\frac{2}{3}$, $\frac{1}{2}$ respectively) before diluting and thoroughly mixing. Increased accuracy is obtained by the use of, a grooved cover-glass (D') which fits over the slightly raised partition dividing the two chambers, which must each be so filled as to present a convex meniscus. The necessary narrowing of the field is secured by cap-diaphragm (Bl'). The average of at least ten determinations are required for accurate work.

Sahli found variations in normal individuals of from 13.7 grams to 17.3 grams per 100 c.c. of blood.

The apparatus is expensive, requires considerable time and care in the making of estimations, and is exclusively an instrument for a well-equipped laboratory.

Sahli's Hæmometer.—A simple and apparently very scientific instrument which has been recently introduced is the Sahli modification of the Gower hæmoglobinometer. Instead of the tinted glass, or gelatin colored with picrocarmine to resemble a definite blood dilution, Sahli uses as a standard the same coloring matter as is present in the tube containing the blood. By acting on blood with 10 times its volume of N/10 HCl, hæmatin hydrochlorate is produced, which gives a brownish-yellow color. In the standard tube, which is sealed, a dilution representing 1% of normal blood is used.

FIG. 55.—Sahli's hæmoglobinometer. (*Greene.*)

To apply this test, pour in N/10 HCl to the mark 10 on the scale of the graduated tube. Add to this 20 cu. mm. of the blood to be examined, drawn up by the capillary pipette provided. So soon as the mixture assumes a clear bright dark brown color, add water drop by drop until the color of the tubes matches. The reading of the height of the aqueous dilution on the scale gives the Hb. reading. The tubes are encased in a vulcanite frame with rectangular apertures. This gives the same optical impression as would plano-parallel glass sides.

The most accurate readings are obtained with artificial light in a dark room but almost as satisfactory comparisons can be obtained with natural light from a window. It is advisable to turn the ruled side around so that one may match colors without being influenced in his determination by the scale.

The apparatus must be kept in a dark place as strong light will change the color of the standard tube. It is recommended that the N/10 HCl be preserved with chloroform.

Tallquist's Hæmoglobin Scale.—This is a small book of specially prepared filter-paper with a color-scale plate of 10 shades of blood colors. These are so tinted as to match blood taken up on a piece of the filter-paper and are graded from 10 to 100. So soon as the blood on the filter-paper has lost its humid gloss, the comparison should be made.

This may be done by shifting the blood-stained piece of filter-paper suddenly from one to the other of the holes cut in each shade—the piece of filter-paper being underneath the color plate; it is better, however, to match the colors with the blood spot against the scale rather than behind a preparation. Grawitz prefers to cut the stained spot from the filter-paper and place it directly on the color scale.

At least a square centimeter of the filter-paper should be stained by the blood. Daylight coming from a window to the rear or at the side should be used in making the comparison. The error with this method is probably not over 10% after a little experience. If the colored plate is not kept in the dark, the tints tend to fade.

Kuttner's Micro-colorimeter.—This apparatus is described under blood sugar. See Fig. 140. The technic and reagents for hæmoglobin estimation are the same as for the Sahli.

To Count Blood-corpuscles

Hæmacytometer.—The instrument almost universally used is the Thoma-Zeiss hæmacytometer. The apparatus consists of two pipettes, one for leukocytes, graduated to give a dilution of 1 to 10 or greater; the other for red cells to give a dilution of 1 to 100 or greater. The white pipette has the mark 11 above the bulb and the red pipette the mark 101. In addition, there is a counting chamber.

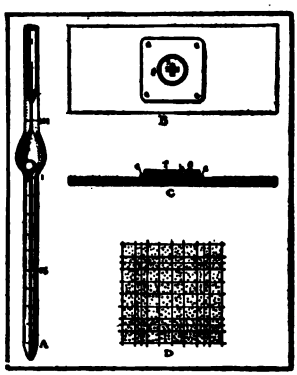


Fig. 56.—Thoma-Zeiss blood counter showing pipette, counting chamber, and ruled field. (*Greene.*)

Counting Chamber.—This consists of a square of glass with a round hole in the center. Occupying the center of this round hole is a circular disc of glass of less diameter, so that an encircling channel is left. The square and the circle of glass are cemented to a heavy glass slide. The surfaces of each are absolutely level and highly polished. That of the circular disc is ruled into squares of varying size and is exactly $\frac{1}{10}$ mm. below the level of the surface of the surrounding glass square.

When a polished plano-parallel cover-glass rests on the shelf, as the outer square glass is termed, there is a space left between its under-surface and the ruled disc of 0.1 mm. The channel around the disc is termed the moat or ditch.

Rulings.—The most desirable rulings are those of Türck, Zappert and Neubauer. In these the entire ruled surface consists of nine large squares, each 1 mm. square. These are subdivided, and in the central large square are to be found the small squares used for averaging the red cells. These small squares are $\frac{1}{20}$ mm. square and are arranged in nine groups of 16 small squares by bordering triple-ruled lines. As the unit in blood counting is the cubic millimeter, if one counted all the white cells lying within one of the large squares (1 mm. square), he would have only counted the cells in a layer one-tenth of the required depth, so that it would be necessary to multiply the number obtained by 10. This product, multiplied by the dilution of the blood, would give the number of white cells in a cubic millimeter of undiluted blood.

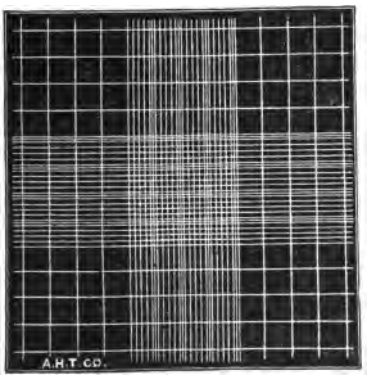


FIG. 57.—Neubauer's ruling.

Bürker Hæmacytometer.—Some workers prefer the *Bürker hæmacytometer*. In this there are two ruled wedge-shaped pieces of glass, separated at their bases, which take the place of the ruled disc of the Thoma apparatus. Two oblong pieces of glass are on either side of the ruled wedges and are 0.1 mm. higher, thus taking the place of the shelf. Clamps fix a cover-glass on these shelves giving a space $\frac{1}{10}$ mm. over the ruled surfaces. The blood is run in by capillarity from the mixing pipette. I gave up this type of counter because the clamps made manipulation awkward.

FIG. 58.—Thoma-Levy counting chamber, Bürker double type with two Neubauer rulings.

Thoma-Levy Chamber.—In the Thoma-Levy modification of the Bürker apparatus the central portion of the slide is cut away and in this depression is cemented a rectangular strip of glass, divided by a central channel. Each half of this strip of glass has a Neubauer ruling on it so that one can make a white count on one side and a red one on the other, simply touching the tip of the red pipette to the space separating the under surface of the cover-glass from the ruled rectangular slips on one side and then with the white pipette repeating the same on the other side. An advantage

of the Thoma-Levy is that the original thickness of the slide makes the shelf on which the cover-glass rests instead of the support being strips on either side of the ruled surfaces and cemented to the slide. The Neubauer ruling is undoubtedly the most satisfactory of the hæmacytometer rulings, its rulings being simpler than those of the Türck system. The unit square in all these hæmacytometers is the small square for counting red blood-cells, $\frac{1}{20}$ mm. square.

Red Cell Counts.—To make a red count: Having a fairly large drop of blood, apply the tip of the 101 pipette to it and, holding the pipette horizontally, carefully and slowly draw up with suction on the rubber tube a column of blood to exactly 0.5 or 1. The variation of $\frac{1}{25}$ inch from the mark would make a difference of almost 3%. If the column goes above 0.5, it can be gently tapped down on a piece of filter-paper until the 0.5 line is cut. Now insert the tip of the pipette into some diluting fluid and, revolving the pipette on its long axis while filling it by suction, you continue until the mark 101 is reached. A variation of $\frac{1}{25}$ inch at this mark would only give an error of about one-thirtieth of 1%. After mixing thoroughly by shaking for one or two minutes, the fluid in the pipette below the bulb is expelled (this, of course, is only diluting fluid). A drop of the diluted blood of a size just sufficient to cover the disc when the cover-glass is adjusted, is then deposited on the disc and the cover-glass applied by a sort of sliding movement, best obtained by using a forceps in one hand assisted by the thumb and index-finger of the other.

Diluting Fluids.—Among diluting fluids Toisson's is probably the best known:

Sodium chloride	1 gram
Sodium sulphate	8 grams
Glycerine	30 c.c.
Distilled water	160 c.c.

Dissolve the sodium chloride and the sodium sulphate in the glycerine water and add sufficient methyl or gentian violet to give a rich violet tint.

Hayem's solution is very satisfactory and is preferred by many workers. It has the following composition: Corrosive sublimate 0.5 gram, Sod. chloride 1 gram, Sod. sulphate 5 grams, 200 c.c. of distilled water.

A 2½% solution of potassium bichromate makes a very satisfactory diluting fluid in the counting of red cells. Recent samples have hæmolyzed red cells.

A salt solution of about 1% strength, tinged with about 1 drop of a saturated alcoholic solution of gentian violet to about 50 c.c., is a good substitute, or the salt solution alone will answer when no white count is to be made at the same time as the red one.

It is important to work quickly in adjusting the cover-glass, or there will be cells settling in the center of the drop from a greater depth than the one which the apposition of the cover-glass makes ($\frac{1}{10}$ mm. deep).

A good preparation should show:

1. Presence of Newton's rings.
2. Absence of air bubbles.
3. Entire surface of ruled disc covered.
4. Equal distribution of cells.

Before counting, about five minutes should be allowed for the settling of the cells.

It will be remembered that the small squares are $\frac{1}{20}$ mm. square. The depth of fluid from upper surface of shelf to lower surface of cover-glass is $\frac{1}{10}$ mm. Hence each space embraced by the small square and the depth of fluid is $\frac{1}{4000}$ of the unit used in estimating number of corpuscles in blood, or 1 cu. mm. ($\frac{1}{20} \times \frac{1}{20} \times \frac{1}{10} = \frac{1}{4000}$). Count 100 of the small squares (this enables one to use decimals). There are nine squares between triple-ruled lines, each containing 16 small squares. Count the number of corpuscles in the 16 small squares contained in upper left-hand triple-ruled square. Put down this count. Next count corpuscles in the adjoining 16 squares. Put down this count. Then in third 16 squares. Put down the number. Now move down to next row of three triple-ruled squares. Count the number of corpuscles in each of the three 16-square spaces and set down the numbers for addition. We have now counted 96 small squares (6×16). Count at any place four additional small squares and add number of blood-cells contained therein to those in the 96 small squares already counted. Divide the sum by 100 or simply point off two decimals. This gives the average for each small square. Multiply this by the dilution and then (as the small square is only $\frac{1}{4000}$ cu. mm.) by 4000. This will give the number of corpuscles in 1 cu. mm. Example: 100 small squares contained 655 red cells. Pointing off, 6.55 equals average number of red cells per small square. Multiply by dilution (200) and then by 4000 (the small square is 4000 times smaller than the unit: 1 cu. mm.)—$6.55 \times 200 = 1310 \times 4000 = 5{,}240{,}000$.

At least 100 small squares, and preferably 200 should be counted. If the blood appears normal, one may simply count the number of red cells in five of the 16 small square spaces (80 small squares). Having added the numbers and multiplying by 10,000, you obtain the number of cells in 1 cu. mm. (Eighty small squares is $\frac{1}{50}$ of the unit of 1 cu. mm., or 4000 small squares. The blood dilution being 1 to 200, we have $50 \times 200 \times$ number of cells in 80 small squares.)

In counting, count corpuscles lying on the lines above and to the right. Do not count those lying on lines below and to the left.

In the small squares count only corpuscles lying in the space or cutting the upper line. This prevents counting the same cell twice.

To Count White Cells.—Draw up the blood in the white pipette to the mark 0.5. Then, still holding the pipette as near the horizontal as possible, because the column of blood tends to fall down in the larger bore, draw up by suction a diluting fluid which will disintegrate the red cells without injuring the whites.

The best fluid is 0.5% of glacial acetic acid in water. This makes the white cells stand out as highly refractile bodies. Some prefer to tinge the fluid with gentian

violet. The 0.5 mark is preferred because it takes a very large drop of blood to fill the tube up to the 1 mark and if there is much of a leukocytosis a 1 to 10 dilution is not sufficient. In leukæmic blood it is better to use the red pipette with the 0.5% acetic acid solution.

The blood having been drawn up to 0.5, we have a dilution of 1 to 20.

Making a preparation, exactly as was done in the case of the red count, we count all of the white cells in one of the large squares (1 sq. mm.). The cross ruling greatly facilitates this. Note the number. Then count a second and a third large square. Strike an average for the large squares counted and multiply this by 10, as the depth of the fluid gives a content equal to only $\frac{1}{10}$ cu. mm. Then multiply by the dilution. Example: First large square 50; second large square 70; third large square 60. Average 60. Then $60 \times 10 \times 20 = 12{,}000$, the number of leukocytes in 1 cu. mm. of blood. The count may be made with a low power ($\frac{2}{3}$-inch objective) as the leukocytes stand out like pearls. It is more accurate, however, to use a higher power, so that pieces of foreign material may be recognized and not enumerated as white cells. If one will accustom himself to comparing the distribution of the leukocytes in a well-made stained dried blood film, prepared according to Ehrlich's cover-glass method, with that in a hæmacytometer preparation, he can readily acquire an experience which will enable him to determine with considerable accuracy the degree of leukocytosis by the examination of a stained cover-glass preparation alone. Furthermore, one can identify the leukocytes in a Giemsa-stained smear with the $\frac{1}{12}$-inch objective. This is specially true of the large mononuclears and transitionals whose increase has such significance in the tropics.

Special diaphragms for the ocular, with a square opening, which just covers one of the large squares of the hæmacytometer (400 small squares) may be purchased or one may cut a square hole from a round piece of stiff paper which rests on the ocular micrometer supports. This is easily measured by noting the number of spaces on the ocular micrometer which equals one side of the square. With such an opening one can count the leukocytes in unruled areas equal to a large square, when the ruling is less convenient than is present in the Türck ruling.

Combined Red and White Counts.—In the absence of a white pipette or when it is desired to make a white count with the same preparation as is used for the red one, especially if the ruling is of the old style (only central ruling and not in nine large squares as with Zappert and Türck), it is advisable to make use of the method of counting by fields. With a Leitz No. 4 ocular and a No. 6 objective, with a tube length of 120 mm., it will be observed that the field so obtained has a diameter of eight small squares. Now, remembering that the area of a circle equals the square of the radius multiplied by π, or 3.1416, we have the following calculation: The diameter being eight small squares, the radius would be four small squares. Squaring the radius, we have 16. This multiplied by 3.1416 gives us 50. This means that every field, with the microscope adjusted as stated, contains 50 of the small squares, or $\frac{1}{80}$ of the unit of 1 cu. mm. of the diluted blood.

By keeping a single red cell in view while moving the mechanical stage from right to left or from above downward, we know that a new field of 50 small squares is brought into view when the circumference of the field cuts this individual cell.

Example: As 2000 small squares would ordinarily be a sufficient number to count for a white count, this would require us to count the number of leukocytes in 40 of the designated microscopic fields (this, of course, is only one-half the unit, hence we should multiply by 2). Counted 40 fields and noted 50 white cells. $50 \times 2 = 100 \times 200$ (the dilution in red pipette) $= 20{,}000$. Consequently 20,000 would represent the number of leukocytes in 1 cu. mm. of the blood examined.

Cleaning Apparatus.—After making a blood count, the hæmacytometer slide should be cleaned with soap and water and then rubbed dry, preferably with an old piece of linen. As the accuracy of the counting chamber depends upon the integrity of the cement, any reagent such as alcohol, xylol, etc., and, in particular, heat, will ruin the instrument. The pipettes should be cleaned by inserting the ends into the tube from a vacuum pump, as a Chapman pump. First draw water or 1% sod. carbonate solution through the pipette, then alcohol, then ether, and finally allow air to pass through to dry the interior. If the interior is stained, use 1% HCl in alcohol. If a vacuum pump is not at hand, a bicycle pump or suction by mouth will answer.

Preparations for the Study of Fresh Blood

Many authorities prefer a fresh-blood specimen to a stained dried smear in the study of parasites of the blood. In malaria in particular there is so much information as to species to be obtained from a fresh specimen that the employment of this method should never be neglected. While waiting for the film to stain one has five or six minutes which could not be better spent than in examining the fresh specimen which only requires a moment to make.

Manson's Method.—Have a perfectly clean cover-glass and slide. Touch the apex of the exuding drop of blood with the cover-glass and drop it on the center of the slide. The blood flows out in a film which exhibits an "empty zone" in the center. Surrounding this we have the "zone of scattered corpuscles," next the "single layer zone" and the "zone of rouleaux" at the periphery. It is well to ring the preparation with vaseline. When desiring to demonstrate the flagellated bodies in malaria, it is well to breathe on the cover-glass just prior to touching the drop of blood.

The method of Ross is very easy of application and gives most satisfactory preparations. Take a perfectly clean slide, and make a vaseline ring or square of the size of the cover-glass. Then, having taken up the blood on the cover-glass, drop it so that its margin rests on the vaseline ring. Gently pressing down the cover-glass on the vaseline makes beautiful preparations which keep for a very long time. If it is desired to study the action of stains on living cells, this method is also applicable. A very practical way to do this is to tinge 0.85% salt solution containing 1% sodium citrate (the same as is used in opsonic work) with methylene azur, gentian violet, or methyl green. With a Wright bulb pipette, take up 1 part of blood, then 1 part of tinted salt solution. Mix them quickly on a slide and then deposit

a small drop of the mixture in the center of the vaseline ring and immediately apply a cover-glass and press down the margins as before. This method will be found of great practical value.

A Method for Making Differential Leukocyte Count in Same Preparation as for White Count

Employ the same technic as in making the ordinary white count but using as a diluting fluid a 1½ or 2% formalin solution to which has been added 1 drop of Giemsa's stain for each c.c. just before making the blood examination.

The best results are obtained when the mixing in the pipette bulb is done immediately after taking up the blood and diluent. Recently I have found it necessary to add enough N/1 NaOH to the commercial formalin to bring it to about +0.75. To do this add to it a few drops of phenolphthalein as an indicator and continue to add a dilute sodium hydrate or sodium carbonate solution until a pink color just develops at room temperature. This corresponds to about +0.75 with boiling titration. The acidity of commercial "Formalins" varies greatly. Of this +0.75 formalin I use 1½% in a ½% glycerine solution instead of water.

The usual technic in making the hæmocytometer preparation is employed using a Türck ruling. Count the leukocytes in the three upper or lower 1 sq. mm. squares, divide by 3 to obtain an average per sq. mm., multiply by 10 for the content of a cubic millimeter and then by 20 for the dilution. (Blood to 0.5; diluent to 11.) This can be done mentally and requires no calculation on paper. Having counted the leukocytes, again go over the same portion of the ruled surface and count the polymorphonuclears and estimate the percentage of these to the total leukocytes. The majority of disrupted cells in a dry-stained preparation are transitionals hence the percentage of polymorphonuclears by this method is lower.

It is unnecessary in such a count to have an assistant; of course, in making a complete differential count it is preferable to have some one tabulate or laboriously to do this one's self.

The red cells are practically diaphanous and not disintegrated as when acetic acid is used as a diluent, consequently it is easy to make out the particular red cell as to size, etc., containing a malarial parasite.

The best results are obtained with a 1/6-inch objective. Higher powers are of course impracticable by reason of the thickness of the cover-glass of the hæmocytometer.

The following are the appearances of the various leukocytes.

Eosinophiles.—In these the bilobed nucleus stains rather faintly and the color is greenish blue. The eosinophile granules show easily as coarse, brickdust-red colored particles.

Polymorphonuclears.—The nucleus stains a deep, rich, pure violet but less intense than that of the small lymphocyte. The shape of the nucleus is typically

three or four lobed but even when of the horseshoe shape of a transitional nucleus is easily recognizable by the intensity of the violet staining. That which makes the polymorphonuclears very easy of differentiation is the distinctness of the cell outlines produced by the fine yellowish granulations in the cytoplasm.

Small Lymphocytes.—The nucleus is perfectly round and stains a deep violet. It is almost impossible to make out any cytoplasmic fringe.

Large Lymphocytes.—The nucleus here is round and of a lighter violet than that of the small lymphocyte. The cytoplasm is blue, nongranular, and sharply defined from the nucleus.

Large Mononuclears.—These show a washed-out, slate-colored nucleus which blends with the gray slate-blue staining of the cytoplasm so that there is an indefiniteness of outline in the more or less irregularly contoured nucleus.

Transitionals.—These show the same characteristics as the large mononuclears, but with a more faintly stained and more indented nucleus. The large mononuclears and transitionals stand out as slate-colored cells. When very much degenerated these cells have a greenish hue.

Mast Cells.—The granulations show as a rich maroon or reddish-violet color.

The young ring forms of malaria show as violet-blue areas in the red cells. When half-grown or approaching the merocyte stage, the containing red cell takes on a faint pink coloration, thereby differentiating it from the noninfected red cells. At the same time the parasite is extruded and has the appearance of a violet-blue body projecting from the margin of the red cell. It is as if a blue body were budding from a pink one. Crescents stand out very distinctly.

It is an easy matter with this method to count the number of trypanosomes or malarial crescents in a cubic millimeter of blood.

Preparation and Staining of Dried Films

When preparations are desired for a differential count, Ehrlich's method of making films is to be preferred, as the different types of leukocytes are more evenly distributed. In making smears by spreading, there is a tendency for the polymorphonuclears to be concentrated at the margin while lymphocytes remain in the central part of the film.

Cover-slip Films.—In *Ehrlich's method* we have perfectly clean dry cover-slips. Take up a small drop of blood without touching the surface of the ear or finger. Drop this cover-glass immediately on a second one and as soon as the blood runs out in a film, draw the two cover-slips apart in a plane parallel to the cover-glasses. Ehrlich uses forceps to hold the cover-glasses to avoid moisture from the fingers, but I find I can work more quickly and satisfactorily with the fingers alone. The method shown in Fig. 59 is a very convenient one. In making malarial smears it is better to wash the finger or ear with soap and water to get rid of all grease and dirt. Then dry thoroughly before puncturing. Alcohol is not so efficient.

Slides and spreaders should be absolutely clean and grease free. Scrubbing with soap and water, thorough rinsing and drying, then subjecting the slide to the flame to make it grease free is satisfactory.

For removing dirt and grease from skin, a mixture of acetone, 40; alcohol, 60; is the best and quickest means. A bottle is kept on hand, with the puncturing needle embedded in the stopper.

For cleaning a slide, nothing equals Bon Ami. Rub up some with the wet finger, rub the slide with the lather until there is a friction squeak; let dry; polish with a clean, dry cloth. This is far better than soap and water, alcohol, ether and flaming combined. Note how a drop of water spreads on a glass so treated.

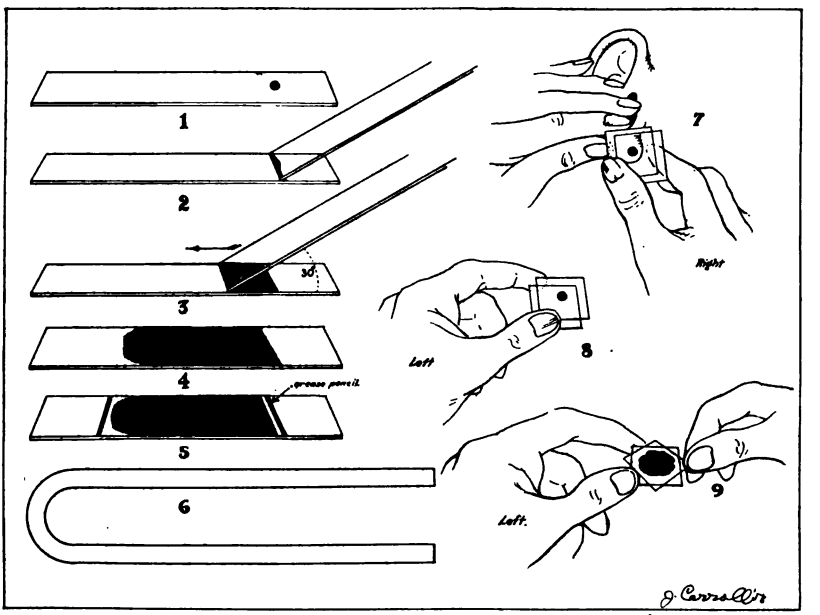


FIG. 59.—1, 2, 3, 4. Making blood smears on slide. 5. Smear ready for staining —grease marks prevent Wright stain from running over slide. 6. U-shaped glass tubing to hold slide in staining. 7. Right hand holding two cover-glasses. One cover-glass is being touched to drop of blood from ear. 8. Cover-glasses transferred to left hand in preparing to place one cover-glass on another and spread film. 9. Separating cover-glasses by sliding one from the other.

Smears on Slides.—Of the various methods of spreading films on slides there is none equal to that described by Daniels. In this the drop of blood is drawn along and not pushed along. The films are even, can be made of any desired thickness by changing the angle of the drawing slide, and there is little liability of crushing pathological cells. Take a small drop of blood on the end of a clean slide. Touch a second slide about ½ inch from end with the drop and as soon as the

blood runs out along the line of the slide end, slide it at an angle of 45° to the other end of the horizontal slide. The blood is pulled or drawn behind the advancing edge of the advancing slide. An angle less than 45° makes a thinner film; one greater, a thicker film.

Instead of a slide a square cover-glass may be used and if the edge be smooth it makes a more satisfactory spreader than the slide.

Instead of the Daniels method I prefer to take up the drop of blood on the slide on which the smear is to be made, about ½ inch from the end. Then apply the spreader slide and so soon as the drop runs along the end of the spreader slide proceed as above described. This method is shown in Fig. 59.

Spreaders.—Of the various methods of making smears by means of cigarette paper, rubber tissue, needles, etc., the best seems to be to take a piece of capillary glass tubing and use this instead of a needle in making the film. There is one advantage about the strip of cigarette paper touched to the drop of blood and drawn out along the slide or cover-glass, and that is that it is almost impossible not to make a working preparation by this method.

Useful Hints.—In the making of smears the chief points are to make the smears as soon after taking the blood as possible and to have slides and cover-glasses scrupulously clean. It is well to flame all slides and cover-glasses which are to be used for blood-work. This is the best method of getting rid of grease.

Thick Film Methods.—Such methods are of the greatest practical value in diagnosis of malarial parasites when in very small numbers in the peripheral circulation. They are also of great value in finding trypanosomes, relapsing fever spirochætes and filarial embryos in the blood. Ruge's method so brings out the polymorphonuclears that such a technic can be used for opsonic index.

Method of Ross.—In this about one-half of a drop of blood is smeared out over a surface about equal to that of a square cover-glass and allowed to dry. It is then flooded with a 0.1% aqueous solution of eosin for about fifteen minutes. The preparation is then gently washed with water and then treated with a polychrome methylene-blue solution. After a few seconds this is carefully washed off and the preparation dried and examined.

Method of James.—James smears out an ordinary drop of blood so that it makes a circular smear about ¾ inch in diameter. This may be easily accomplished with a spatulate wooden toothpick. When dry, treat the blood smear with alcohol containing HCl. (Alcohol 50 c.c. HCl 10 drops) until the hæmoglobin is dissolved out. Then wash thoroughly in water for five or ten minutes. Allow to dry and then stain as ordinarily with the Wright or Giemsa stain.

Ruge's Method.—The best thick film method is that of Ruge. After the blood has dried well, gently move the slide about in a glass containing a 2% solution of

formalin to which has been added 1% of glacial acetic acid. After laking is complete, as shown by disappearance of brown color, treat the slide in the same way in a glass of tap water to remove all traces of acid. Next wash very gently in distilled water and stain with dilute Giemsa (1 drop to 1 c.c.) for twenty to thirty minutes. Wash in water and allow to dry without heat or blotting paper.

Some workers prefer to stain the dried thick smear for one hour in a jar containing dilute Giemsa stain (1 to 40) without previous fixation or dehæmoglobinization.

At present, I make my thick films by taking up a moderately large loopful from the exuding drop of the puncture wound. This is deposited at one end of the slide and from it three or four more daubs are made in succession toward the other end of the slide. These daubs are quickly smeared out before coagulation takes place in the first daub.

With all thick film methods it is extremely important to have thorough drying of the smear before dehæmoglobinizing or staining. This ordinarily requires one or two hours in the air or twenty to thirty minutes in the incubator. It is particularly important in working with such smears, although holding for ordinary smears, to protect them from flies, ants, etc., as such insects will eat up the smear in a few minutes if left exposed.

Fixation of Film.—In Wright's, Leishman's, and other similar stains the methyl-alcohol solvent causes the fixation. In staining with Giema's stain, Ehrlich's triacid, hæmatoxylin and eosin, Smith's formol fuchsin, and with thionin, separate fixation is necessary. For Giemsa and thionin, either absolute alcohol (ten to fifteen minutes), or methyl alcohol (two to five minutes) answer well.

Formalin vapor, for five to ten seconds, is also used for fixation. For Ehrlich's triacid, hæmatoxylin and eosin and formol fuchsin, heat gives the best results. The best method is to place the films in an oven provided with a thermometer. Raise the temperature of the oven to 135°C. and then remove the burner. After the oven has cooled, take out the fixed slides or slips.

Some prefer to place a crystal of urea on the slide, then hold it over the flame until the urea melts. This shows that a temperature between 130° and 135°C. has been reached.

One of the handiest methods is to drop a few drops of 95% alcohol on the slide or cover-glass. Allow this to flow over the entire surface; then get rid of the excess of alcohol by touching the edge to a piece of filter-paper for a second or two. Then light the remaining alcohol film from the flame and allow the burning alcohol to burn itself out. A chemical fixation which gives good fixation for hæmatoxylin and triacid stains (not equal to heat) is a modification of Zenker's fluid (Whitney). To Müller's fluid, which is potassium bichromate 2 grams, sodium sulphate 1 gram, and water 100 c.c., add 5 grams of bichloride of mercury and 5 c.c. of nitric acid (C. P.). Fixation is obtained in five seconds.

When using corrosive sublimate fixation one should after thorough washing in water treat the film with Gram's iodine solution for about two minutes and then wash with 70% alcohol until the yellow color of the film disappears. Methyl alcohol for two minutes is satisfactory. (See Staining of Protozoa.)

Staining Blood-films.—As separate staining with eosin and methylene blue rarely gives good preparations and as the modifications of the Romanowsky stain recommended are easy to make and employ, and give much more information, the separate method of staining is not recommended. The most satisfactory single stain is thionin.

Rees' Thionin Solution.—Take of thionin 1.5 grams, alcohol 10 c.c., aqueous solution of carbolic acid (5%) 100 c.c. Keep this as a stock solution. It should be at least two weeks old before using. For use, filter off 5 c.c. and make up to 20 c.c. with water.

1. Fix films (a) by heat, (b) by alcohol and ether, or (c) preferably by 1% formalin in 95% alcohol for one minute.

2. Stain for from ten to twenty minutes. Wash and mount. Malarial parasites are stained purplish; nuclei or leukocytes, blue; red cells, faint greenish blue.

Ehrlich's Triacid or Triple Stain.—There are required:

1. Sat. aq. sol. orange G. (Dissolve 3 grams in 50 c.c. water.)
2. Sat. aq. sol. acid fuchsin. (Dissolve 10 grams in 50 c.c. water.)
3. Sat. aq. sol. methyl green. (Dissolve 10 grams in 50 c.c. water.)

These three solutions may be kept as stock solutions. They keep well in the dark. To make the stain, add 9 c.c. of No. 2 (acid fuchsin) to 18 c.c. of No. 1 (orange G.). After they are mixed thoroughly, add 20 c.c. of No. 3 (methyl green). Then after the first three ingredients are well mixed, add 5 c.c of glycerine. Mix, then add 15 c.c. of alcohol; again mix, and finally add 30 c.c. of distilled water. Keep the mixed stain about one week before using. The best fixatives are heat and Whitney's modified Zenker. To use, stain films from two to five minutes; then wash and mount. The triacid stain is a good tissue stain. The objections to the triacid stain are that it does not stain malarial parasites or mast cells and that failure to obtain good results is of frequent occurrence.

Romanowsky Stains

Wright's Method.—The stain is made by adding 1 gram of methylene blue (Grubler) to 100 c.c. of a ½% solution of sodium bicarbonate in water. This mixture is heated for one hour in an Arnold sterilizer. The flask containing the alkaline methylene-blue solution should be of such size and shape that the depth of the fluid does not exceed 2½ inches. When cool, add to the methylene-blue solution 500 c.c. of a 1 to 1000 eosin solution (yellow eosin, water soluble). Add the eosin solution slowly, stirring constantly until the blue color is lost and the mixture becomes purple with a yellow metallic luster on the surface, and there is formed a finely granular black precipitate. Collect this precipitate on a filter-paper and when thoroughly dry (dry in the incubator at 38°C.) dissolve 0.3 gram in 100 c.c. of pure

methyl alcohol (acetone free). Wright lately has recommended using 0.1 to 60 c.c. methyl alcohol. This constitutes the stock solution. For use filter off 20 c.c. and add to the filtrate 5 c.c. of methyl alcohol.

A modification by Balch is very satisfactory. In this method instead of polychroming the methylene blue with sodium bicarbonate and heat, the method of Borrel is used. Dissolve 1 gram of methylene blue in 100 c.c. of distilled water. Next dissolve 0.5 gram of silver nitrate in 50 c.c. of distilled water. To the silver solution add a 2 to 5% caustic soda solution until the silver oxide is completely precipitated. Wash the precipitated silver oxide several times with distilled water. This is best accomplished by pouring the wash-water on the heavy black precipitate in the flask, agitating, then decanting and again pouring on water. After removing all excess of alkali by repeated washings, add the methylene-blue solution to the precipitated silver oxide in the flask. Allow to stand about ten days, occasionally shaking until a purplish color develops. The process may be hastened in an incubator. When polychroming is complete, filter off and add to the filtrate the 1 to 1000 eosin solution and proceed exactly as with Wright's stain.

In Leishman's method the polychroming is accomplished by adding 1 gram of methylene blue to 100 c.c. of a ½% solution of sodium carbonate. This is kept at 65°C. for twelve hours and allowed to stand at room temperature for ten days before the eosin solution is added. The succeeding steps are as for Wright's stain.

Wilson's Stain.—This stain has the same polychroming principle as for the Balch stain. The quantities of the reagents, however, are somewhat different. In using the stain the method of procedure is exactly the same as for the Balch stain. We have recently been using this stain and it would seem that it has more of the rich-staining characteristics of the Giemsa than the Balch shows.

The first thing to obtain is the silver oxide. To prepare this dissolve 2 grams of silver nitrate in 15 c.c. of distilled water; then add to this 250 c.c. of a freshly prepared solution of calcium hydrate. Shake the mixture well and when all the precipitate has settled collect it on a filter, washing several times with distilled water to get rid of alkali. The precipitate collected on the filter-paper is then dried in a hot air oven (not to exceed 80°C.). Prepare the lime water by U. S. P. directions. Instead of carrying out the above technic we adopt the same method as used with the Balch stain, using the moist, freshly prepared silver oxide, instead of the dried product.

Two solutions are prepared:

Solution I. To the moist silver oxide, or the dried product if preferred, add 2 grams of methylene blue dissolved in 200 c.c. of a ½% solution of sodium bicarbonate in distilled water.

Gently boil this silver oxide methylene-blue mixture in a rather deep porcelain dish for twenty minutes, stirring occasionally. Pour off about one-third of the contents of the dish into a 200 c.c. graduated cylinder. Add to the contents of the dish an amount of boiling distilled water, equal to that poured into the cylinder, and boil again for twenty minutes. Then pour one-third the contents of the porcelain dish into the cylinder. Then boil the remaining half of the contents of the dish for another twenty minutes, not adding additional water. Now pour the contents of the dish into the cylinder and make up the total volume with water to

200 c.c. Filter through a coarse filter into a 500 c.c. beaker and immediately add to it.

Solution II. For this solution dissolve 1 gram of yellow water soluble eosin in 200 c.c. distilled water. Allow the mixture of the two solutions to stand about thirty minutes and then filter, collecting the precipitate on a hard filter-paper. For collecting the precipitate either for the Balch or Wilson stain we use a Buchner porcelain filter with stationary perforated disc. The stem of the filter is inserted into a doubly perforated rubber stopper which fits into a large flask. Glass tubing is passed into the other perforation and the filtration flask is connected up with a filter pump. The precipitate may be dried in an incubator or hot air oven at 60°C. The yield of dry precipitate is about 1.7 grams. For the staining fluid dissolve 0.2 gram in 50 c.c. pure methyl alcohol.

In all Romanowsky methods distilled water should be used. If not obtainable, the best substitute is rain-water collected in the open and not from a roof.

If the yellow color in water in a test-tube to which has been added a small pinch of hæmatoxylin does not change to blue in from one to five minutes it is too acid and should be treated with a 1% sod. carb. sol. until it does show blue. Alkaline waters are less easy to correct.

Technic of Staining.—Method of staining:

1. Make films and air dry.

2. Cover dry film preparation with the methyl alcohol stain for one minute (to fix).

3. Add water to the stain on the cover-glass or slide, drop by drop, until a yellow metallic scum begins to form. It is advisable to add the drops of water rapidly in order to eliminate precipitates on the stained film. Practically, we may add 1 drop of water for every drop of stain used.

4. Wash thoroughly in water until the film has a pinkish tint.

5. Dry with filter-paper and mount. The stained preparation is less apt to show foreign material and damage if one allows the film to dry without blotting. In a moist atmosphere one may dry the film high over the flame but any near contact with the heat of the flame is injurious.

Stained Cells.—Red cells are stained orange to pink; nuclei, shades of violet; eosinophile granules, red; neutrophile granules, yellow to lilac; blood platelets, purplish; malarial parasites, blue; chromatin, metallic red to rose pink.

By using a 1% solution of sodium carbonate instead of distilled water for diluting the Wright stain various spirochætes even treponemata are quite intensely stained.

The bottle in which the methyl alcohol solution is kept must be tightly stoppered with a cork stopper and kept in the dark. Any evaporation of the alcohol interferes with proper fixation of the blood film and the light affects the delicate stain.

Giemsa's Modification of the Romanowsky Method.—This is one of the most perfect of the modifications. The objection is that greater time in staining films is required than with the Wright or Leishman method and the stain is very expensive.

Take of Azur II eosin 0.3 gram. Azur II 0.08 gram.

Dissolve this amount of dry powder in 25 c.c. of pure anhydrous glycerine at 60°C. Then add 25 c.c. of methyl alcohol at the same temperature. Allow the glycerine methyl-alcohol solution to stand over night and then filter. This is the stock stain. To use: Dilute 1 c.c. with 10 to 15 c.c. of distilled water. If 1 to 1000 potassium carbonate solution is used instead of water it stains more deeply.

The alkaline diluent is used to obtain the course stippling in malignant tertian (Maurer's clefts). Having fixed the smear with methyl alcohol for one to five minutes, pour on the diluted stain, and after fifteen to thirty minutes wash off and continue washing with distilled water until the film has a slight pink tinge. For *Treponema pallidum* stain from two to twelve hours.

While the Romanowsky methods are more satisfactory for differential counts and for the demonstration of the malarial parasites, and especially for differentiating species, yet by reason of the liability to deterioration in the tropics of methylene blue the hæmatoxylin methods may be preferable. Many workers in blood-work and cytodiagnosis prefer the hæmatoxylin.

1. Fix the film either by heat, with methyl alcohol for two minutes or with Whitney's fixative. Heat is to be preferred.

2. Stain with Meyer's hemalum or Delafield's hæmatoxylin for from five to fifteen minutes according to the stain. Frequently three minutes will be found sufficient. To make the hemalum, dissolve 0.5 gram of hæmatin in 25 c.c. of 95% alcohol. Next dissolve 25 grams of ammonia alum in 500 c.c. of distilled water. Mix the two solutions and allow to ripen for a few days. The stain should be satisfactory in two or three days.

To make Delafield's hæmatoxylin, dissolve 1 gram of hæmatoxylin crystals in 6 c.c. of 95% alcohol. Add this to 100 c.c. of saturated aqueous solution of ammonia alum. After exposure to light for a week, the color changes to a deep blue purple. Add to this ripened stain 25 c.c. of glycerine and 25 c.c. of methyl alcohol and, after it has stood for about two days, filter. The stain should be filtered from time to time as a sediment forms. This makes a stock solution which should be diluted 10 to 15 times with water when staining.

Mink's Modification of Unna's Hæmatoxylin

Hæmatoxylin	1 gram
Alum	8 grams.
Sulphur (sublimed)	1 gram.
Glycerine	30 c.c.
Alcohol	50 c.c.
Water	100 c.c.

Dissolve the hæmatoxylin in the glycerine in a mortar. Dissolve the alum in the water and add it to the glycerine hæmatoxylin in the mortar. Then add the sulphur and the alcohol. The solution ripens in about three to four days. Allow the sediment to remain in the bottom of the bottle containing the stain and filter off small quantities as needed.

3. Wash for two to five minutes in tap water to develop the hæmatoxylin color.

4. Stain either with a 1 to 1000 aqueous solution of eosin or with a one-half of 1% eosin solution in 70% alcohol. The eosin staining only requires fifteen to thirty seconds.

5. Wash and examine.

Coagulation Rate, Viscosity and Iodophilia

Iodophilia.—This reaction is supposed to be due to the presence of glycogen, especially in the polymorphonuclears, in suppurative conditions. At present this test is rather discredited.

It has been stated that a differentiation between the joint involvement in gonorrhœal infection and in articular rheumatism may be made from iodophilia being present in the gonococcus infection.

Make blood-smears on cover-glasses as usual, and after they dry, but without fixation, mount them in a drop of the following solution:

Iodine	1 part.
Potassium iodide	3 parts.
Gum arabic	50 parts.
Water	100 parts.

Small brown masses in the polymorphonuclears indicate a positive iodophilia.

Viscosity of the Blood.—This is estimated by observing the relative height to which blood rises in capillary tubes as compared with water, and normally varies from three to five. The higher the hæmoglobin content the greater the viscosity. Viscosity is high in arterio-sclerosis and diabetic coma, low in the anæmias of nephritis.

Coagulation Rate of Blood.—This determination is of value in connection with operations on jaundiced patients.

Besides hæmophilia, in which the coagulation rate (vein) is delayed an hour or more it is also observed in certain cases of pernicious anæmia and the leukæmias.

An accelerated coagulation rate in typhoid fever indicates the occurrence of thrombosis.

Wright's coagulometer is a standard instrument but is cumbersome.

A simple method of determining the rate is to take a piece of capillary glass tubing and hold it downward from the puncture to let it fill for 3 or 4 inches. Then at intervals of thirty seconds scratch with a file the capillary tubing at short distances and break off between the fingers. When coagulation has taken place a long worm-like coagulum is obtained. Normally coagulation occurs in about three to four minutes, when the temperature is that of the hand in which the tubes are conveniently held.

Rudolf recommends placing the tubes in metal tube containers in a Thermos bottle at 20°C. He gives the normal coagulation rate for this temperature as eight minutes, while at a temperature below this the period is lengthened. Age and sex do not influence the rate. Sabrazes, the originator of this method found no appreciable variation in tubes from 0.8 to 1.2 mm. diameter.

It has now been well established that the proper source of the blood for testing coagulation rate is from the vein.

We now attach a piece of glass tubing, one end of which has been drawn out into a capillary tube, to a hypodermic needle by a short rubber connection. See Fig. 141. Insert the needle into the vein and fill the capillary tube. File off as above. From the vein, at room temperature the coagulation rate is from six to eight minutes. From the ear or finger three to four minutes.

In Bürker's test you mix a drop of blood and a drop of distilled water on a slide and with a capillary tube sealed off at the end stir the mixture every half minute. So soon as fibrin threads appear you have coagulation.

Specific Gravity of the Blood

Hammerschlag has a method for the determination of the Hb. percentage based upon the specific gravity of the blood.

In this method a mixture of benzol and chloroform is made of a specific gravity of about 1050. A medium size drop of blood is then taken up with a pipette and introduced below the surface of the mixture, carefully avoiding production of bubbles. Variation in temperature introduces a very appreciable error. If it sinks add more chloroform from a dropping bottle, if it tends to rise, more benzol. The mixture in which the drop of blood tends to remain stationary, near the top of the mixed benzol and chloroform, has the same specific gravity as that of the blood. This is determined by an accurately graduated hydrometer. The normal average specific gravity for men is 1059, for women 1056. A table, giving the Hb. percentage corresponding to the specific gravity, accompanies the outfit.

Eykmann controls the specific gravity of the drop of blood by adding a drop of salt solution made to have a similar specific gravity.

The specific gravity is reduced in all anæmias, especially chlorosis; in nephritis with œdema as well as in most cachectic states.

In these latter the Hb. percentage may be normal.

Specific Gravity in Cholera.—To determine the necessity for intravenous infusion in cholera Rogers has recently recommended the employment of small bottles containing aqueous solution of glycerine with specific gravities varying from 1048 to 1070, increasing the specific gravity in each successive bottle by 2°.

An accurate urinometer will suffice to determine the specific gravity. Drops of blood from the cholera patient are deposited at the center of the surface of the fluid in the bottles from a capillary pipette. If the specific gravity of the blood is 1062 at least a liter of saline or sodium bicarbonate solution is needed. If 1066 at least

2 liters. Formerly he estimated the indications by blood-pressure considering a pressure of 80 in Europeans or of 70 in natives as indicating intravenous injections.

Tests for Agglutination and Hæmolysis of the Red Cells (Transfusion)

Blood transfusion has been so simplified by the various technics of indirect transfusion, using citrated solutions for preventing the coagulation of the blood, that simple laboratory methods for determining the suitability of the blood of donors is of immense importance.

In war surgery, with the treatment of cases in which there has been great loss of blood, it is a therapeutic measure that must be considered. Blood transfusion is indicated not only in traumatic hæmorrhages but, as well, in those anæmias resulting from prolonged loss of blood in carcinoma, alimentary tract ulcerations, etc. This measure is also advocated in pernicious anæmia. The best results seem to follow its use in the treatment of melena neonatorum. Although alkali injection of sodium bicarbonate is the measure usually recommended in severe cases of blackwater fever yet blood transfusion should be kept in mind.

In the selection of a donor for blood transfusion it is always necessary to try his red cells against the serum of the recipient as well as the patient's red cells against the serum of the donor, in order to prove the absence of hæmolyzing or agglutinating bodies.

Certain persons have isohæmolysins in their blood which dissolve the red cells of other persons and in paroxysmal hæmolgobinuria autohæmolysins may be present which can destroy the patient's own red cells. This autohæmolysin seems operative only when a low temperature is followed by a high one. When hæmoglobinæmia exists the liver converts it into bile pigment, causing bilious stools and jaundice. If one-sixth of the red cells are destroyed hæmoglobinuria results.

It is well to do a Wassermann test on donors.

In the following tables the grouping of bloods is according to the Moss ones, which differ from those of Ottenburg.

	Serums of groups			
Cells of groups	1	2	3	4
1	−	+	+	+
2	−	−	+	+
3	−	+	−	+
4	−	−	−	−

	Cells of groups			
Serum of groups	1	2	3	4
1	−	−	−	−
2	+	−	+	−
3	+	+	−	−
4	+	+	+	−

According to Moss 10% of bloods belong to group 1, 40% to group 2, 7% to group 3 and 43% to group 4. Karsner has recently tested the bloods of 1000 individuals with the following findings: For group 1, 3.1%; group 2, 42.4%; group 3, 8.3% and group 4, 46.2% .

Before transfusing carry out the following tests:

From a vein take about 1 c.c. of blood in a centrifuge tube containing 1% of sod. citrate salt solution; then shift the stopper of the blood system to a dry centrifuge tube and draw into it about 3 or 4 c.c. of blood. Throw down the citrated blood, pipette off the supernatant fluid and wash the sediment with normal saline.

Again pipette off the saline after centrifuging and make a 10% emulsion of the red-cell sediment in normal saline.

Centrifuge the coagulated blood in the other tube and collect the serum which separates from the clot.

Carry out these procedures for both donor and recipient.

Tests: 1. In a small test-tube deposit 1 drop of the donor's 10% red-cell emulsion and then add 4 drops of the recipient's serum.

2. Treat similarly 1 drop of the recipient's red-cell emulsion with 4 drops of the donor's serum.

3. Treat 1 drop of donor's red-cell emulsion with 4 drops of his serum.

4. Treat 1 drop of recipient's red-cell emulsion with 4 drops of his serum. Finally add 1 c.c. of salt solution to each of the four tubes, shake gently and place in incubator for two hours.

Tests 3 and 4 should fail to show either agglutination or hæmolysis.

Some prefer to keep the tubes over night in ice-box after the preliminary examination following incubation. In addition to the four tubes noted above it is well to also have two control tubes, 5 and 6. In tube 5 put 1 drop of donor's red-cell emulsion and 4 drops of normal saline. In tube 6 put 1 drop of donee's red-cell emulsion and 4 drops of saline.

Karsner has recently written an article highly recommending Lee's technic for use in military hospitals.

For the regular carrying out of this method one should keep on hand the sera of individuals belonging to groups 2 and 3. To carry out the tests prepare a suspension of the donor's red cells by dropping 2 or 3 drops of his blood into 1 c.c. of citrated salt solution. Deposit a platinum loopful of standard serum 2 on a slide and emulsify in it a loopful of the donor's red-cell suspension. The agglutination can be observed with a high power magnifying glass or the ⅔-inch objective. Agglutination, when it occurs, is usually complete in five to fifteen minutes. Repeat test with serum 3. It would seem safe to use the cells of any donor of group 4 as such cells are not agglutinated by the sera of any group. It is, however, advisable to try to obtain a donor whose blood belongs to the same group as the donee. When standard sera 2 and 3 are not on hand one may use the following *emergency method* of Lee:

"A small amount of blood is collected from a patient (1 c.c. from the ear or finger is sufficient), and allowed to clot. The serum is then obtained. One drop of

this serum is placed on a slide and mixed with a drop of suspension of blood of the donor taken into 1.5 per cent. citrate solution. (A few drops of blood are taken into approximately 10 times the amount of 1.5 citrate solution and shaken. It is very important that the blood be dropped directly into the citrate, and should not be partially coagulated.) The test will appear in a few moments, and is best examined under the microscope, where, in the event of a positive test, marked agglutination will be evident. The test will also be evident macroscopically. In the event of a negative test it is a wise precaution to raise the cover-glass, and after making sure that the serum and cells are well mixed, to examine the preparation again. The only possible source of confusion is the appearance of rouleaux of the red corpuscles, indicating a too thick emulsion. If the test is negative, transfusion may be regarded as entirely safe."

Substitutes for Blood.—There are practical objections to bleeding donors from a standpoint of military efficiency. As a substitute for human blood the latest recommendation is to use solutions of gum arabic. This solution can be readily sterilized by boiling whereas the gelatin solutions require prolonged autoclaving on account of the spore bearers which gelatin usually contains. Then too there is no danger from anaphylaxis with gum arabic solutions, these being protein free. At the British front they have been using 2% solutions of gum arabic (acacia). Others have recommended a 4 or 5% solution. Rous and Wilson state that the lesser percentages may suffice except in extreme cases where a percentage of acacia of 7% is indicated for prolonged effect on the blood-pressure. While the 2 or 4% solutions keep up the blood-pressure longer than saline injections, the action of which on the blood-pressure is transient, yet even these acacia solutions do not give the prolonged effect obtained with a 7% acacia solution. It is not necessary to have the acacia solution of the same viscosity as the blood.

Occult Blood

When the presence of blood cannot be recognized by macroscopical or microscopical methods (occult blood) we must resort to spectroscopic or chemical tests. It is in connection with blood in the fæces that these tests for occult blood are chiefly called for. Before making such tests on fæces it is advisable to have the patient on a meat-free and green-vegetable-free diet for two or three days. It is chiefly in carcinoma or ulcerations of the gastro-intestinal tract that such examinations of the fæces are required.

Hæmin Crystal Test (Teichmann).—Prepare a solution of 0.1 gram each of KI, KBr, and KCL in 100 c.c. of acetic acid. This is a stable solution. Mix some of the material with a few drops of the solution on a slide, apply a cover-glass and warm the material until bubbles begin to appear (gentle steaming), then examine for dark-brown crystals.

Blood in the Urine.—The most rapid method of detection is by using the microspectroscope. An ordinary hand spectroscope will answer however.

Donogany's test is very satisfactory. To 10 c.c. of urine add 1 c.c. ammonium sulphide solution and 1 c.c. of pyridin. The urine will assume a more or less deep orange color according to its blood content. The spectrum of alkaline methæmoglobin or hæmochromogen will be obtained. See illustrations under urine.

In making the guaiac or other tests it is a good plan to repeatedly filter the blood-containing urine through the filter. Then touch a spot on the moist filter with the guaiac or benzidin solution and then finally drop on this so treated spot a drop or two of hydrogen peroxide solution.

Blood in Fæces or Gastric Contents.—Take 5 grams of fæces and rub it up thoroughly in a mortar with 15 c.c. of a mixture of equal parts of alcohol, glacial acetic acid and ether. Filter through an unmoistened pleated filter-paper repeatedly until only 3 to 4 c.c. remain of the filtrate. The fæces filtrate can be first tested chemically by depositing a few drops in the center of three or four circles of white filter-paper placed in a Petri dish or upon an ordinary white plate.

Weber Guaiac Test.—The moistened spot is then treated with a few drops of a freshly prepared alcoholic solution of guaiac resin (about ½ gram of guaiac resin is broken up into small fragments and shaken up in about 3 c.c. of alcohol) and finally there is dropped upon the spot a few drops of a solution of hydrogen peroxide. Waves of blue color extending out into the moistened filter-paper show a positive test for blood.

Benzidin Test.—For the benzidin test pour on this fæces filtrate-moistened filter-paper a few drops of the following solution: 2 c.c. of a saturated alcoholic solution of benzidin 2 c.c. of solution of peroxide of hydrogen and 2 drops of glacial acetic acid. (Blue.)

If the *aloin test* is preferred we treat the filtrate-moistened filter-paper with a few drops of a 3% solution of aloin in 70% alcohol and then treating the spot with hydrogen peroxide solution. Brick-red color.

Phenolphthalin Test.—The phenolphthalin test is an extremely delicate one and will show the pink color at times with certain specimens of water, hence one should always make a control using the reagents without addition of the suspected blood material.

In my opinion it has great value as a negative test.

Take 2 c.c. of the ether alcohol acetic-acid filtrate and dilute with 7 or 8 c.c. water. Neutralize the acidity with sodium hydrate. Then add 1 c.c. of the phenolphthalin reagent, mix and finally add several drops of 1 to 10 dilution of peroxide of hydrogen and note the formation of a decided rose-pink coloration, varying in depth according to the amount of occult blood.

To prepare the reagent dissolve 1 or 2 grams phenolphthalein and 25 grams KOH in 100 c.c. distilled water. Add 10 grams powdered zinc and heat gently until solution is decolorized. Phenolphthalin is a reduction product of phenolphthalein.

Some modification of the *Wagner "dry test"* is now preferred by laboratory workers. A little powdered benzidine is picked up with a knife-blade point (about twice the size of an ordinary pin's head) and to this is added 1 c.c. of glacial acetic acid and 10 drops of 3% peroxide of hydrogen solution. The reagent keeps for only a few hours. To carry out the test smear out a piece of fæces about the size of a match head on a piece of white paper and drop on about 2 or 3 drops of the reagent. The greenish-blue color should show in a few seconds.

More Reliable is the Spectroscopic Test.—For this we take about 3 c.c. of the concentrated ether, acetic acid, alcohol fæcal filtrate and add to it 2 c.c. of pyridin. Then add not more than 2 to 3 drops of ammonium sulphide solution. (The ammonium sulphide solution should be kept in an amber-colored, glass-stoppered bottle. The solution should be freshly prepared every ten days.)

Examine the solution, contained in a small test-tube, with the spectroscope and the two absorption bands of methæmoglobin-alkaline (hæmochromogen), between D and E, show a positive blood test. Comparison should be made with fresh blood, in which the absorption band in the yellow is nearer line D (oxyhæmoglobin spectrum).

The great trouble about the spectroscopic test is that it will only show the presence of quite large amounts of blood. *It is by no means a delicate test.*

Acidosis and Methods for its Determination

Everyone is familiar with that form of respiratory disturbance associated with diabetic coma, known as Kussmaul's air hunger. Here we have hyperpnœa, a form of dyspnœa typically without cyanosis, and the best clinical evidence of acidosis.

Reduced alkalinity of the blood would be a better expression than acidosis because even a neutral reaction of the blood would be incompatible with maintenance of life.

Acidosis is an important consideration in alimentary tract disturbances of infants and children, as in infantile diarrhœas or cyclical vomiting of children. It is not infrequent in the pneumonias of children. In adults we must keep the possibility of the occurrence of acidosis in mind in the vomiting and eclampsia of pregnancy, in salicylate poisoning, following chloroform anæsthesia, and in chronic nephritis as well as in many infectious diseases.

Sellard's alkaline treatment for the acidosis of cholera is a measure of the utmost value.

Starvation, whether the result of gastric ulcer, gastric carcinoma or otherwise, is a recognized cause of acidosis. The insistence upon one-sided diets in children is often the cause of increased acid content of the blood and such causes are not absent in many of the dietary treatments of diseases of adults, whether in the height of the disease or during convalescence.

From the laboratory standpoint acidosis is usually recognized by an increase in the urinary acetone bodies or by noting an increase in the ammonia quotient due to demands upon the alkali for neutralization of the increased acid production. Ace-

tone, diacetic acid and β-oxybutyric acid (acetone bodies) come from abnormal metabolism of fats. When not formed in excessive amounts the two acids are converted into acetone and appear in the urine as such. With more marked production acetone formation falls behind and diacetic acid appears or, with still more marked acid production, β-oxybutyric acid.

These acids, of themselves, seem harmless and their injurious action is connected with abstraction of alkali from the blood.

There are some who think lactic acid may be formed from abnormal metabolism of carbohydrates and that certain cases of acidosis failing to show increase of acetone bodies may be connected with lactic acid increase. Of course, retention of acids of normal metabolism would also cause acidosis. To prevent this the organism utilizes a sufficient amount of the ammonia from protein or rather amino-acid metabolism instead of further changing it into urea. So that an increased ammonia quotient may signify that nature has control of the abnormal acid production.

In the acidosis connected with chronic nephritis the preformed ammonia may be lower than normal indicating some defect in the mechanism of ammonia neutralization of acids.

Acetone bodies can come from proteins as well as fats. Whatever may be the explanation of abnormal formation of acetone bodies it seems to be associated with inability of the organism to obtain sugar for its tissues, hence the therapeutic value of giving glucose in acidosis conditions where the trouble is carbohydrate deficiency. Glucose administration is frequently combined with alkali treatment in acidosis. Of course, where the trouble is an inability on the part of the cells to utilize the sugar, which may be circulating in greatly increased amounts in the blood, from lack of pancreatic internal secretion (as in diabetes), sugar injection would have no effect on this abnormal acid production.

Even in ordinary metabolism great amounts of acid are produced but these are eliminated normally by way of lungs as well as urine. In this connection a failure on the part of the kidneys to remove acid would result in acid retention and, if sufficiently marked, acidosis.

Besides the phosphoric and sulphuric acid produced in the metabolism of the phosphorus and sulphur of proteids we have enormous amounts of carbonic acid formed in the tissues.

The alkalinity of the blood is maintained by the bicarbonate of soda, by sodium and potassium phosphates and to some extent proteins can neutralize acids. The carbonic acid is taken up by the bicarbonate of the blood and gotten rid of as CO^2 by the lungs, without any loss of sodium bicarbonate.

Other metabolic acids, however, cause a loss of sodium bicarbonate (and along with this the other blood alkalis) so that the determination of the lowering of this salt in the blood indicates an acidosis. This may be carried out by van Slyke's method described below.

It has been found that the alveolar CO^2 falls with a fall in the plasma bicarbonate. (This however does not hold with cardio-respiratory cases.) Therefore by determining the CO^2 content of the expired air upon forced expiration we can judge as to reduction of blood carbonate and of course acidosis.

Sellards' method for determination of serum acidosis is quite simple and reliable. To carry out the test we add 1 c.c. of serum, drop by drop, to 25 c.c. of absolute alcohol (it is very important to secure a neutral alcohol and I have found that of Merck satisfactory). This precipitates the protein which is the factor interfering with a sharp end reaction. After filtering we add 3 or 4 drops of neutralized phenolphthalein solution to the filtrate and evaporate the alcohol in a porcelain dish on a water-bath. Every piece of apparatus must be perfectly dry and the steam vapor of the bath quite low to avoid the taking up by the alcohol of water. In normal cases the dark pinkish tinge of the sediment, after evaporation, will remain at least one hour, while with cases showing increased acidosis the reddish tinging of the sediment disappears in a few minutes. An electric bath is desirable.

A very simple way of determining bicarbonate diminution is the test for tolerance of alkalis. The giving of 5 grams of bicarbonate of soda to a normal person on a mixed diet will bring about an alkaline urine. Boiling the urine will bring out the alkalinity to litmus more sharply.

These amounts are increased until the urine becomes alkaline. In some cases of acidosis massive doses of bicarbonate, as 150 grams in one or two days fail to produce an alkaline urine.

Plasma Bicarbonate.—Van Slyke has devised an apparatus for the determination of the carbon dioxide capacity of oxalate plasma.

He uses 1 c.c. of the plasma which is shaken with air containing 6% of CO^2 and is introduced into the following apparatus. A 50 c.c. pipette-shaped apparatus is provided at top and bottom with three-way stopcocks and connected with a bulb of mercury. The pipette is first filled with mercury and the plasma having been introduced followed by 1 c.c. of water and 0.5 c.c. of N/1 acid the mercury is withdrawn from the pipette by lowering the bulb reservoir, thus creating a Torricellian vacuum. The CO^2 escapes from the solution as the result of a few minutes shaking and the watery solution is drawn off from the lower cock. The mercury is again made to fill the pipette by the other entrance of the three-way cock at the bottom and rises to the level of the CO^2. This volume is read off in the calibrated upper stem of the pipette (calibrated in 0.02 c.c. divisions) and the calculations made accordingly.

Normal serum binds about 75% of its volume of CO^2 while in acidosis figures as low as 20% may be obtained.

For the determination of the CO^2 content of alveolar air the apparatus of Fredericia is quite practical. In this the volume of CO^2 in 100 c.c. of alveolar air is obtained. In such methods temperature plays so important a part in estimating volume that it is hardly applicable except in the hands of one accustomed to gas analysis.

We have constructed an apparatus, using a Sedgwick aerobioscope as an air chamber and fitting the ends with glass stopcocks. The patient having expired, to get rid of air in upper air passages, then with forced expiration fills the 200 c.c. chamber of the aerobioscope, immediately afterward closing the stopcocks. We then introduce 5 c.c. of N/1 KOH, shaking at intervals for one-half hour to allow absorption of CO^2 by the KOH. We then add a few drops of phenolphthalein as indicator and titrate in the aerobioscope chamber the loss in alkalinity of the KOH, using N/1 HCl. (For hydrogen-ion concentration method see Appendix.)

Besides the tests for serum acidosis of Sellards and that for carbon dioxide content of alveolar air we should also determine ammonia nitrogen output as well as its quotient.

Urinary tests for acidity and acetone bodies content of the urine should also be made as well as that for alkali tolerance. These tests are all very simple and can be easily carried out by any well-trained laboratory worker.

CHAPTER XIV

NORMAL AND PATHOLOGICAL BLOOD

In considering what may be termed normal blood, it must be borne in mind that the normal varies for men, women, and children:

	Hb.	Red cells	Leukocytes
Men,	90 to 110%,	5 to 5½ million,	7500
Women,	80 to 100%,	4½ to 5 million,	7500
Children,	70 to 80%,	4½ to 5 million,	9000

Color Index

This is obtained by dividing the percentage of the hæmoglobin by the percentage of red cells, 5,000,000 red cells being considered as 100%.

To obtain the percentage of red cells it is only necessary to multiply the two extreme figures to the left by two. Thus if a count showed the presence of 1,700,000 red cells, the percentage would be $34(17\times2=34)$. If the Hb. percentage in this case were 50; then the color index would be $50\div34$, or 1.4.

In normal blood the color index is, approximately, 1.

In anæmias we have three types of color index: 1. The pernicious anæmia type, which is above 1. Here we have a greater reduction in red cells than we have of the hæmoglobin content of each cell. 2. The normal type, when both red cells and hæmoglobin are proportionally decreased, as in anæmia following hæmorrhage. 3. The chlorotic type. Here there is a great decrease in hæmoglobin percentage, but only a moderate decrease in the number of red cells. Hence the color index is only a fraction of 1. For example, in a case of chlorosis we have 40% of hæmoglobin and 90% of red cells, $40\div90=0.4$.

One can judge fairly well the approximate color index by noting the character of the staining of the red cells. This is faint in bloods of low color index and deeper than normal in cells in a case with high color index.

Red Cells

In considering the corpuscular richness of a specimen of blood, it must be remembered that this does not necessarily bear any relation to the quantity of blood in the body. Thus, a more or less bloodless-

looking individual, the total quantity of whose blood is greatly reduced, may, notwithstanding, give a normal red count. In examining a specimen of peripheral blood we get a qualitative, not a quantitative result.

Normally, we have an increase in red cells in those living at high altitudes. An altitude of 2000 feet may increase the red count about 200,000, and a height of 6000 feet about 500,000. Profuse sweats and diarrhœas also increase the red count. Pathologically, in chronic polycythemia with cyanosis and splenic enlargement, we have a red count of about 10,000,000. In cyanosis from heart disease, etc., and in Addison's disease there is also an increase in red cells.

The normal red cell or erythrocyte measures about 7.5μ in diameter and 2μ in thickness. It is nonnucleated and normally stains with acid dyes, taking the pink of eosin or the orange of orange G. If larger, 10 to 20μ, it is called a macrocyte; if smaller, 3 to 6μ, a microcyte. It is usually stated that the life of a red cell after leaving the bone-marrow is about thirty days. On this basis $\frac{1}{30}$ of the total number of red cells are destroyed each day. The old and damaged red cells are destroyed by the macrophages of various internal organs, chiefly the spleen. The bone marrow normally or even after considerable loss of blood pours out normal type red cells. If the loss of blood be great or continuous then various types of immature red cells are given off. Such cells show regenerative power of the bone-marrow. Should these pathological cells not appear in the blood of a case of severe anæmia, showing lack of regenerative power of the bone-marrow we apply the term *aplastic* to such an anæmia.

Anisocytosis is a term applied to a condition where marked variation in size of the red cells occurs.

Macrocytes (10–18μ) are rather indicative of severe forms of anæmia, the microcytes (1–6μ), of less grave types. When the red cell is distorted in shape, it is called a poikilocyte. Care must be exercised that distorted shapes are not due to faulty technic. Crenation and vacuolation of red cells are marked in poorly prepared specimens.

In addition to variation in size and shape, we also have pathological variation in staining affinities.

Achromia.—This is characterized by pallor of the central portion of the stained red cell. It also shows as a central vacuolation in fresh blood and is apt to deceive one in the anæmic blood of malaria.

Polychromatophilia.—This shows itself by red cells taking a brownish to a dirty blue tint, as is frequently seen in immature red cells, especially nucleated ones.

Granular basophilic degeneration (also termed punctate basophilia and stippling) refers to the presence of blue dots in the pink background of stained red cells. It is found in many severe anæmias, as

pernicious anæmia, the leukæmias, malarial cachexia, etc. It is very characteristic of lead poisoning.

This punctate basophilia (Grawitz granules) often coexists with polychromatophilia (polychromasia). It is not determined whether these granules mark degenerated cells or immature ones.

Nuclear Fragments.—Bloods showing nucleated reds may have red cells which fail to show a definite nucleus but possess irregular fragments of nuclear material.

Howell's Bodies.—These bodies stain a pinkish or bluish color with Giemsa's stain. They are larger than the Grawitz granules and are quite round and well differentiated from the red cell.

There are rarely more than two present in a cell. They are most often noted in punctate basophilia blood.

Chromatin Dust.—These are single or double small red granules usually seen at the periphery of the red cell. They are smaller than Howell's bodies.

The nucleated red cell, while normal for the marrow, is always pathological for the blood of the peripheral circulation.

Normoblasts have the diameter of a normal red cell. The nucleus is round and stains intensely with basic dyes, often appearing almost black. Another characteristic is that it frequently appears as does the setting in a ring. Some give the term microblast to smaller nucleated forms. In normoblasts the red cell proper stains normally. The megaloblasts not only have a greater diameter than the normoblast, but the nucleus is poor in chromatin, stains less intensely and is less distinctly outlined. Instead of being round, the nucleus is irregular and may be trefoil in shape. The cytoplasm surrounding the nucleus shows polychromatophilia. This contrasted with the pure blue of the lymphocytes should differentiate.

Normoblasts are found in secondary anæmias, and especially in myelogenous leukæmia. Megaloblasts are peculiarly characteristic of pernicious anæmia. Enormous megaloblasts are sometimes termed gigantoblasts.

In aplastic anæmia (a severe type of pernicious anæmia), in contrast to ordinary pernicious anæmia, nucleated reds are very rarely found. There is also very little poikilocytosis, and the color index is about normal. It is a rare, rapidly fatal anæmia, particularly of young women.

It does not show remissions, runs a rapid course, and is attended with a marked increase of lymphocytes. The bone marrow of the femur is pinkish yellow and homogeneous. In this disease there is failure on the part of the bone marrow to

make cells while in pernicious anæmia proper there is increase in production but also increase in destruction of red cells.

The term *leukanæmia* has been employed to describe conditions which partake of the characteristics of pernicious anæmia and leukæmia.

White Cells

Owing to the conflicting views as to origin, nature, and functions of the various leukocytes, their classification is in a state of confusion.

As regards the appearance of the cells, this of course varies as the stain used, and it requires considerable experience for a single individual to be able to positively recognize the difference between a lymphocyte and a large mononuclear when one specimen is stained with a Romanowsky stain, another with Ehrlich's triacid, and a third with a hæmatoxylin and eosin. This, of course, is intensified when different persons adhere to the method of staining which they prefer and are at a loss to appreciate differences which are brought out by some other stain used by some other person. Even with the same stain used with different specimens of blood we find the staining characteristics of various leukocytes imperceptibly merging the one into the other, so that at times it is impossible for one, even with his own standard of differentiation, to be sure whether he is dealing with a lymphocyte or a large mononuclear. The difficulty is even greater when we deal with Türck's irritation forms and with myelocytes.

Without going into the various granule stainings so thoroughly brought out by Ehrlich, we shall immediately take up the question of a practical classification for use in making a differential count.

As the Romanowsky method of staining (Wright, Leishman, or Giemsa) gives us information not yielded by either hæmatoxylin and eosin or the triacid, the points of differentiation to be referred to in that which follows is with blood so stained.

In considering the staining affinities of different parts of the leukocytes, it is convenient to divide such into basic ones, acid ones, and those which may be said to be on the border line between these—the so-called neutrophilic affinities.

With Wright's stain we have the eosinophile or oxyphile affinity of the granules of eosinophiles for acid dyes, in this case eosin. The nuclei and basophile granules have affinities in greater or less degree for basic stains (the blue and the violet shading resulting from methylene blue as modified by polychroming). With the granules in the cytoplasm of the polymorphonuclears and neutrophilic myelocytes, and to a less extent in the transitional, we have a staining which merges into a yellowish red on the one extreme and into a lilac on the other. As a standard, neutrophilic granules should be a mean of these extremes.

Not only by reason of the authority of Ehrlich, but because such a division gives all variations, which can then be combined by one preferring a simpler classification, it would seem proper to divide the

normal leukocytes into hyaline and granular cells. Of the former we have the lymphocytes, the large mononuclears and the transitionals. Of the latter the polymorphonuclears, the eosinophiles and the mast cells.

Hyaline Leukocytes

1. **Lymphocytes.**—As a rule, the cells of this type are about the diameter of a red cell. They have origin in lymphadenoid tissue. The nucleus is generally quite round but may show one or more bulging processes.

The large lymphocytes are rare in the blood of adults but make up about 10% of the leukocytes of young children. In making a count it is best to group large and small lymphocytes under one heading but for distinction we may divide them into:

(*a*) *Small Lymphocytes.*—These are small round cells about the size of a red corpuscle with a large centrally placed, deeply violet staining nucleus and a narrow zone of cytoplasm. This cytoplasm may not be more than a mere crescentic fringe. This is the type of lymphocyte which makes up the greater proportion of the leukocytes in chronic lymphatic leukæmia. At times these cells seem to be composed of nucleus alone.

This nucleus contains one or two nucleoli. These cells are often called small mononuclears. In infants they make up more than 50% of the leukocyte percentage.

(*b*) *Large Lymphocytes.*—These are of the same type as small lymphocytes, but possessing more cytoplasm. The nucleus, while round and taking a fairly deep rich violet stain, does not stain so deeply as the nucleus of the small lymphocytes.

The cytoplasm is a clear, translucent, pure blue. It may contain pinkish granules known as azur granules, but these are of rather large size and do not mar the glass-like appearance. They are from 12 to 15μ in diameter and are common in children. In the acute lymphatic leukæmias they at times predominate.

These cells are similar to the cells of the germinal centers of the lymphatic glands.

2. **Large Mononuclears.**—These are large round or oval cells with a nucleus which has lost the richness of violet staining of the lymphocyte nucleus. The nucleus is furthermore frequently irregular in outline or may show the commencing indentation of the transitional nucleus.

There is not that sharp distinction between nucleus and cytoplasm that exists in the lymphocytes. The cytoplasm of the large mononuclear gives the impression of opacity, as if it were frosted glass instead of clear glass. This is due to very fine

granules rather like the larger neutrophilic granules of polynuclears. The neutrophile mottling which begins to appear causes a disappearance of the pure blue character of the cytoplasm of the lymphocyte. It is principally by the washed-out staining of the nucleus and the opaque lilac of the cytoplasm that we differentiate them from the lymphocytes. They greatly resemble Türck's irritation forms or plasma cells and may be confused with myelocytes.

3. **Transitionals.**—These appear as but a later stage in the decay of the large mononuclears; the nucleus is more indented, frequently horseshoe-shaped, and has a washed-out violet shade of less intensity than that of the large mononuclears. These are the cells so often disrupted in smears. A good name for the fragmented cell smears is "smudges." The old view that the transitional was the precursor of the polymorphonuclear has few advocates at the present time.

While it may be convenient to consider hyaline cells as representing different stages in development, yet from a standpoint of immunity this is untenable. The large mononuclears and transitionals are the cells in which we find certain animal cells and pigment phagocytized, as is the case in malaria. These cells are the macrophages of Metchnikoff and are probably derived from the bone marrow.

In the tropics one of the most important points in a differential count is the matter of an increase in the large mononuclears and transitionals, both of which seem to respond to the same stimulus, which is most commonly malaria but may also be from other protozoal infections.

From a practical standpoint I always group them together and as a matter of fact it is difficult to separate a large mononuclear showing considerable irregularity of nucleus from a transitional with less marked nuclear indentation.

Origin of Hyaline Leukocytes.—The lymphocytes take origin from the lymphoid tissue, and very probably the large lymphocyte is a younger, more immature cell than the small lymphocyte.

Ehrlich and Naegeli regard the large mononuclears as of myeloid origin while Pappenheim considers them to belong to the group of lymphocytes.

A normal percentage of large mononuclears and transitionals combined should not exceed about 4%.

Granule Containing Leukocytes

In addition to the series of leukocytes just considered we have present normally in the blood three types of granular cells distinguished according to the staining affinity of their granules. These are:

1. **Polymorphonuclear Leukocytes.**—This cell normally constitutes the greater proportion of the leukocytes. It is an amœboid, actively phagocytic cell, about 10 or 12μ in diameter, and is the microphage of Metchnikoff.

Bacteria are actively phagocytized by this cell, and it is the cell concerned in determining the opsonic power of blood to various bacteria. It has fine lilac granules which are termed neutrophilic (epsilon granules). The single nucleus is rich in chromatin and is lobose like the kernel of an English walnut; frequently it resembles the letter z. These cells are derived from the neutrophilic myelocytes of the bone marrow. It is in these cells that the glycogen, or iodophil granules, appear in certain suppurative conditions.

Arneth Index.—A great deal of interest has been aroused in the so-called Arneth index, especially in connection with prognosis in tuberculosis and various pyogenic infections. The basis of the test is that polymorphonuclears showing only one or two nuclear nodes are considered immature while those having three, four or five nuclear nodes possess greater phagocytic power.

A normal distribution is as follows:

Class I	Class II	Class III	Class IV	Class V
6%	35%	42%	16%	1%

To obtain the Arneth index add to the sum of the polymorphonuclear percentages of cells containing one and two nodes one-half of the percentage of those having three nodes. In the above we have as the normal Arneth index 62.

In an advanced case of tuberculosis we might have an index of 79, obtained as follows:

Class I	Class II	Class III	Class IV	Class V
20%	45%	28%	6%	1%

2. **Eosinophile Leukocytes.**—These are very striking cells with coarse granules staining brilliantly pink, the eosinophile, oxyphile, or acidophile granules (alpha granules of Ehrlich). The cells are a little larger than the polymorphonuclears.

The normal eosinophile is to be distinguished from the eosinophilic myelocyte by possessing two distinct lobes in the nucleus. At times we find three nuclei. The nucleus of the myelocyte is round. The eosinophile is the cell so frequently increased in infections by intestinal animal parasites.

3. **Mast Cells.**—These also have coarse granules, but they stain a deep violet blue. Hence they are basophile granules (gamma granules). In fresh blood these granules do not show up very well, thus they can be distinguished from the highly refractile granules of the eosinophile. The trilobed nucleus stains less intensely than the granules. As a rule, the mast cell is about the size of a polymorphonuclear.

In a differential count of normal blood we find about the following percentages.

Polymorphonuclears,	65 to 70%,	about 5000 per cu. mm.
Small lymphocytes,	20 to 30%,	about 1500 per cu. mm.
Large lymphocytes,	2 to 6%,	about 200 per cu. mm.
Large mononuclears,	1 to 2%,	about 100 per cu. mm.
Transitionals,	2 to 4%,	about 200 per cu. mm.
Eosinophiles,	1 to 2%,	about 100 per cu. mm.
Mast cells,	¼ to ½%,	about 25 per cu. mm.

NOTE: The lymphocyte percentage of infants is about 60.

DIFFERENTIAL COUNT

In making a differential count I would recommend the following from the directions of Schilling-Torgau.

It will be remembered that considerable interest was raised a few years ago in what was termed the Arneth index. In this the more normal, more mature, better resisting polymorphonuclears were considered to have three or four lobes to the nuclear structure, even occasionally five. The immature cells had only one or at most two lobes to the nucleus. The index was obtained by adding the percentages of cells showing one and two lobes to one-half the percentage of those with three lobes. As will be understood a high percentage of these immature cells was unfavorable in prognosis. These cells are graded from left to right I, II, III, IV, V, as to separate masses in the nucleus, so that when the percentage is shoved or displaced to the left it indicates an increase in the immature cells.

Schilling-Torgau divides his polymorphonuclears into 1. the myelocyte which is always of course a pathological cell; 2. the immature form polymorphonuclear. In this there is a close resemblance to the neutrophile myelocyte but there is a nuclear indentation instead of the round nucleus of the myelocyte. It is this cell which often puzzles us as to whether to regard it as a true myelocyte. It is the metamyelocyte of many authorities. 3. Between the mature or segmented polymorphonuclear and the immature one or metamyelocyte we have what may be designated the band form nucleated one. These show the type of nucleus which one is familiar with in the nucleus of the transitional. 4. The mature, multilobed or segmented nucleus of the typical polymorphonuclear.

It would seem that if all laboratory workers would agree upon some single method of recording differential counts it would be advantageous.

In the differential count he not only divides up the polymorphonuclears but makes no separation of small from large lymphocytes. Although I have always divided lymphocytes into large and small ones I believe it unnecessary and impractical and shall henceforth group all such cells in one grouping. The statement that large mononuclears and transitionals are cells of a similar origin, type and significance has always been my idea.

SCHEME OF SCHILLING-TORGAU

Type of cell		Normal percentage	Percentage moderate sepsis (W.C. 14,000)
1. Mast cells.		1	1.0
2. Eosinophiles.		3	1.5
3. Neutrophiles.	a. myelocytes.	0	0.5
	b. immature forms (metamyelocytes).	0	5.0
	c. bandform (Stabkernige).	4	13.5
	d. multilobed (Segmentkernige).	63	64.0
4. Lymphocytes.		23	10.5
5. Large mononuclears and transitionals.		6	4.0

PATHOLOGICAL LEUKOCYTES

The leukocytes which are found in the peripheral circulation only in pathological conditions are:

1. **Neutrophilic Myelocytes.**—The common type is a large cell with a large centrally placed, feebly staining nucleus.

This may be recognized by the difficulty of distinguishing the nucleus from the cytoplasm, there being no sharp line separating these parts of the cell. They imperceptibly merge into one another. They differ from a large mononuclear in that the cytoplasm is distinctly dotted with neutrophile granules and that we cannot make out a distinct line of separation of a slightly irregular or indented nucleus from the surrounding slightly neutrophilic cytoplasm. Cornil has described a very large myelocyte with eccentrically placed nucleus and neutrophilic granules.

Myelocytes are at times found with both basophilic and neutrophilic granules, and may rarely be seen to have all three kinds of granules on a single myelocyte, acidophile, basophile, and neutrophile.

2. **Eosinophilic Myelocytes.**—These can be distinguished from normal eosinophiles by their possessing a single round nucleus, not bilobed. These myelocytes may be as large as a normal eosinophile, but frequently are no larger than a red cell.

The neutrophile myelocyte is characteristic of spleno-myelogenous leukæmia, the eosinophile one of myelogenic leukæmia. The occurrence of an occasional neutrophilic myelocyte is frequently noted in conditions having a leukocytosis. In diphtheria their presence in numbers is of bad prognostic import. Myelocytes are of diagnostic importance in metastases of malignant tumors.

3. **The Irritation Cell of Türck or Pathological Myeloblast.**—This cell has a faintly staining, eccentrically placed nucleus, and a dark opaque blue, frequently vacuolated, cytoplasm. They are usually recorded as large mononuclears. Türck supposed them to appear in the circulation as the result of bone-marrow irritation.

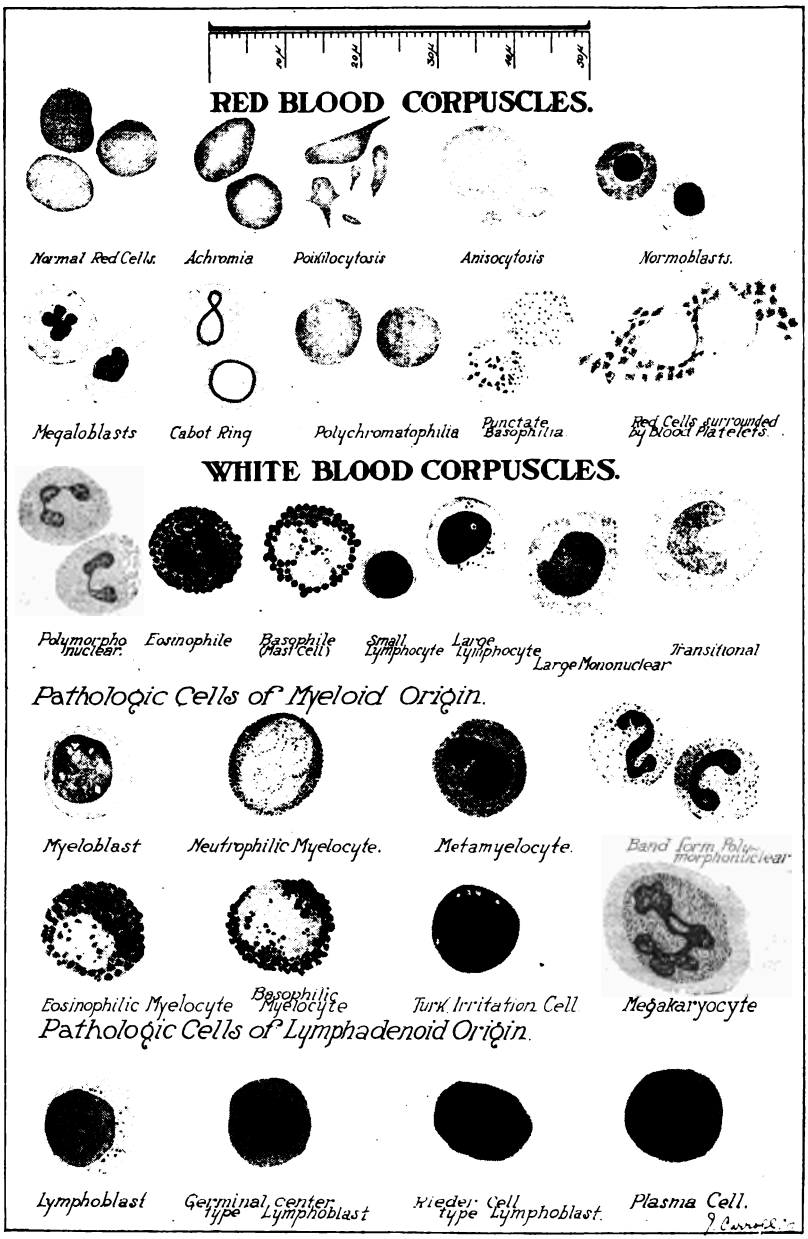


FIG. 60.—Normal and pathological blood cells.

4. **Myeloblasts.**—These cells are found in myeloid leukæmia and though often mistaken for lymphocytes they are of marrow origin. They are the lymphoid cells of the marrow and are the parent cells of myelocytes.

The nucleus stains more intensely than that of the large mononuclear and the cytoplasm is more deeply blue stained than that of the large lymphocyte. They also show three or four nucleoli in the nucleus. There is no perinuclear clear zone and no azure granules in cytoplasm as have the pathological large lymphocytes.

Pyronin methyl-green staining is best for demonstrating the nuclei.

5. **Pathological Large Lymphocytes.**—These are, as a rule, much larger than normal large lymphocytes and show poorer staining of both nucleus and cytoplasm. The nuclei often show the appearance of division into two or more lobes, thus showing the characteristics of Rieder cells.

They may be confused with large mononuclears but are considered to be derived from the germinal centers of various lymphoid tissues. They are found in leukæmic and pseudo-leukæmic conditions.

6. **Megakaryocytes.**—These are the giant cells of the bone marrow and are but rarely found in the blood. The nucleus is gnarled.

7. **Plasma Cells.**—These are very rarely found in the blood and resemble lymphocytes but have an eccentric nucleus of wheel-spoke appearance. Some authorities designate Türck cells plasma cells.

These plasma cells are of lymphadenoid and not of myeloid origin as are Türck cells.

Blood Platelets

These are normally present in blood in the number of about 350,000 per cubic millimeter. They disintegrate very quickly after the blood is withdrawn. Wright has demonstrated that they are pinched-off projections of giant cells of the bone marrow. They consist only of protoplasm, no nuclear material. They do not contain hæmoglobin. In conditions where giant cells are less abundant, as in pernicious anæmia, the blood platelets are less abundant. In myelogenous leukæmia they are very abundant. They vary in size from 2 to 5μ according as a larger or smaller pseudopod of a giant cell has been broken off. Stained with Wright's stain, they are more purplish than blue and show thread-like projections. They are often mistaken for the protozoal causes of various diseases. Especially are they confused with malarial parasites when lying on a red cell. The blood plate has no brick-red chromatic material; it is purplish rather than blue, and has no pigment grains. It is advisable to compare these isolated blood plates with the larger or smaller aggregations scattered

about the smears. In this way their true character is apparent. Blood platelets are readily approximately estimated in a well-stained Romanowsky preparation. Then too the variations in size show up well. The Wright and Kinnicutt method is the standard one for their estimation. In this the blood is drawn up to 1 and a diluting fluid consisting of 2 parts of 1 to 300 brilliant cresyl blue and 3 parts of potassium cyanide (1 to 1400) is drawn up to 100. Blood platelets are increased in leukæmias and following hæmorrhages as well as in trichinosis. In pernicious anæmia and severe infectious diseases they are diminished. In addition to blood platelets, which in fresh blood can only be observed when a fixative is used, we have other confusing bodies.

The *hæmokonia of Müller* are small, highly refractile bodies showing active oscillatory movement. They are supposed to be cast-off granules of eosinophiles or other leukocytes, or possibly derived from nuclei. As this blood dust or hæmokonia is found in a marked degree in lipæmia it may be that the particles are fat. It is interesting that this lipæmia is absent after the taking of large quantities of fat in cases with serious pancreatic trouble. The serum of a normal individual is rather turbid after slight indulgence in butter. Pinched-off fragments of red cells may also appear as possible protozoal bodies.

Leukopenia

This is a term used to designate a reduction in the normal number of leukocytes. A leukocyte count of 5000 would represent a slight leukopenia; one of 2000, a marked leukopenia. In the latter stages of typhoid, and in acute miliary tuberculosis, we expect a moderate leukopenia. Glandular tuberculosis may give a very marked leukopenia. Tuberculous peritonitis will show moderate leukopenia or a normal count.

The leukopenia of typhoid is moderate and at times preceded in the first few days by a moderate neutrophile leukocytosis. Later on we have a decided increase in the lymphocytes. A marked diminution or absence of eosinophiles is so characteristic that any increase in eosinophilic percentage negatives a diagnosis of typhoid.

Paratyphoid gives a similar blood picture.

Chronic alcoholism and chronic arsenic poisoning cause a reduction in the number of the white cells. Pernicious anæmia, especially the aplastic type, shows a marked leukopenia, as is also the case with Banti's disease. Two tropical diseases, kala-azar and dengue, show a marked leukopenia, the counts often being below 2500. During the apyrexial period of malaria we may have a white count of 5000.

It has recently been claimed that a leukopenia with a coincident marked reduction in the lymphocytes is characteristic of measles and that this occurs several days before the Koplik spots appear.

Kocher notes that in exophthalmic goiter the leukocyte count is considerably diminished and that the polymorphonuclears are not much more than one-half the

usual percentage while the percentage of the lymphocytes is almost double the normal.

X-ray treatment tends to destroy leukocytes in the exposed region, especially polymorphonuclears. The small lymphocytes are least affected.

EOSINOPHILIA

Where the eosinophiles are increased to 5%, we have a moderate eosinophilia. In some cases of infection with intestinal parasites, especially hook-worms, but also from other parasites, as round and whip-worms, we may have an eosinophilia of 30 to 50%. In Guam, among the natives, it is difficult to find an eosinophile count under 15%. The eosinophilia tends to disappear when the anæmia becomes very severe.

Echinococcus infection has an eosinophilia which disappears when the cyst is removed. Continuance of the eosinophilia indicates that all cysts were not gotten rid of.

The eosinophilia of trichinosis is best known, and a combination of this blood finding with fever and marked pains of muscles, would justify the excision of a piece of muscle for examination for encysted embryos.

In true asthma eosinophilia is marked, and its absence is of value in indicating other causes for the condition. Certain skin diseases, especially pemphigus, show eosinophilia. Blastomycoses are usually found to show eosinophile increase.

Eczema and psoriasis are not apt to give more than 3 or 4% eosinophiles. A rather high degree of eosinophilia is found in mycosis fungoides.

Scabies also gives an eosinophilia.

The proportion of eosinophiles in the blood of children is greater than in that of adults.

At the height of the disease there may be a rather marked eosinophilia in scarlet fever.

Increase of both eosinophiles and mast cells is found in myelogenous leukæmia.

An eosinophilia tends to appear following splenectomy. With a Wright stain showing acid tendencies one may count polymorphonuclears as eosinophiles unless noting smaller size of granules.

Hodgkin's disease may show a marked increase of eosinophiles.

Leukocytosis

It is to an increase in the polymorphonuclears that this term is usually applied, the term lymphocytosis or eosinophilia being employed where white cells of eosinophile or lymphocyte nature are increased. We have physiological leukocytosis in the latter weeks of pregnancy, also in the new-born, and in connection with digestion.

Pathological Leukocytosis.—Pneumonia. In this disease we have a leukocytosis of 20,000 to 30,000 or higher. The eosinophiles are almost absent. A normal leukocyte count in pneumonia makes a prognosis unfavorable.

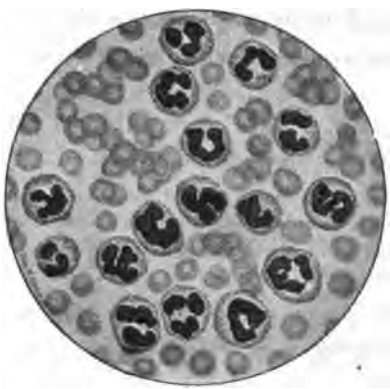


Fig. 61.—Leukocytosis (40,000); sixteen polymorphonuclears in field. (*Cabot.*)

The leukocyte count drops about the time of the crisis, and with the reappearance of eosinophiles is a favorable sign.

Toxæmic conditions as uræmia, diabetic coma and poisoning by CO^2 tend to show a leukocytosis.

Septic processes. The leukocyte count is of great value, especially when we obtain a leukocytosis with 80 to 90% of polymorphonuclears, as in appendicitis, cholecystitis, or other suppurative conditions. Read article under the blood cultures, Chap. XXXI. A marked leukocytosis is of diagnostic importance in acute ulcerative endocarditis provided it is not fulminant in type.

According to Cabot, leukocytosis varies in infections as follows:

1. Severe infection—good resistance; early, marked and persistent leukocytosis.
2. Slight infection—slight resistance; leukocytosis present, but not marked.

3. In fulminating infections we may have no increase in whites, but a higher percentage of polymorphonuclears.

4. Slight infection and good resistance may not be productive of leukocytosis.

It is in connection with the question of operation in appendicitis or similar conditions that the matter of a leukocyte count is of prime importance. If there be a leukocytosis but with less than 75% of polymorphonuclears it indicates an infection of little virulence or a walled-off process with an exacerbation. It is difficult to form an opinion when the polymorphonuclears are under 80%. Leukocytosis with polymorphonuclear percentage of 85 to 90 indicates immediate operation; percentages over 90 point to peritonitis and if with such percentages of polymorphonuclears there is absence of leukocytosis the prognosis is grave.

The blood of cases with malignant tumors tends to show a moderate leukocytosis except in epithelioma of the skin. When a cancer is ulcerating quite a high white count may be obtained.

Spirochæte fevers, as relapsing fever, may give a leukocytosis of from 12,000 to 17,000.

Smallpox, especially at time of pustulation, plague, scarlet fever, and liver abscess give a leukocytosis of from 12,000 to 15,000.

Smallpox often shows a very large percentage of very characteristic large mononuclears. In this disease a leukopenia precedes the leucocytosis.

The leukopenia and lymphocyte increase in measles are important points in differentiating it from scarlatina.

Influenza shows a leukopenia at first, then a leukocytosis and, following the fall in fever, a second lowering.

With meningitis, except in the tuberculous type, counts of 25,000 are not unusual, in abscess of the brain the white count rarely exceeds 15,000.

Poliomyelitis and polioencephalitis give a slight leukocytosis during the febrile accession.

Erysipelas and epidemic cerebrospinal meningitis also give a leukocytosis of from 15,000 to 20,000. In malignant diseases we sometimes have a moderate leukocytosis. Rogers states that in liver abscess, with a leukocytosis of 15,000 to 20,000, we have only about 75 to 77% of polymorphonuclears—there being also a moderate increase in the percentage of large mononuclears.

Drugs such as antipyrin may give a leukocytosis. The leukocyte increase of pilocarpine is rather a lymphocytosis. Cinnamate of soda, sodium nucleate, bacterin injections and turpentine have been used in kala-azar to increase leukocytes.

Lymphocytosis

Of course, the disease in which we have the most marked lymphocytosis is lymphatic leukæmia.

The lymphocytosis of typhoid fever has been taken up under leukopenia.

Whooping-cough may give a lymphocytosis of 20,000 to 30,000.

Young children have normally an excessive proportion of lymphocytes even to a reversal of the polymorphonuclear-lymphocyte relation of adults. This is apt to be particularly marked in hereditary syphilis. Enlarged tonsils may give rise to a lymphocytosis of 10,000 to 15,000 when more than 50% of the white cells will be lymphocytes. Rickets and scurvy give a lymphocytosis.

In pellagra there is a moderate lymphocytosis, averaging 34% in about a normal count.

Varicella and mumps may also give an increase in the percentage of lymphocytes.

Malta fever is a disease which may show quite a lymphocyte increase, this going with a reduction in polymorphonuclears.

Increased Large Mononuclears

In tropical work we combine the large mononuclears and transitionals in a differential count. They are the phagocytes of animal cells or parasites. The disease in which their increase is best recognized is malaria and an increase to 15% where the blood shows moderate leukopenia is very significant. The melaniferous leukocytes of malaria are cells of this type.

Other protozoal infections, as kala-azar, trypanosomiasis and amœbiasis cause it. Filterable virus diseases may show a mononuclear increase, thus yellow fever and dengue both give an increase about the fifth or sixth day.

In Banti's disease there is an increase in cells of this type and a transitional increase is reported for Hodgkin's disease.

Diseases in which there is a Normal Leukocyte Count

Uncomplicated tuberculosis, influenza, Malta fever, measles, trypanosomiasis, malaria, syphilis, and chlorosis.

In malaria we have a leukocytosis at the time of the rigor, while during the apyrexial period there is a moderate leukopenia. In malaria we have a marked increase in the percentage of the large mononuclears and transitionals. These may form from 25% to 35% of the leukocytes. (When bearing particles of pigment they are known as melaniferous leukocytes—macrophages which have ingested malarial material.) In dengue, at the time of the terminal rash, we may have as great a percentage of large mononuclears. In this disease, however, we have a great diminution of polymorphonuclears from the start (25 to 40%). Instead of a large mononuclear we have at the onset a lymphocytic increase. There is an increase of large mononuclears in trypanosomiasis.

The white count is about normal in uncinariasis (Ashford's average was 7800). Some have reported a leukopenia in severe cases.

While eosinophilia is the most marked feature in hook-worm disease yet in very severe cases it may be absent.

The Primary Anæmias

Chlorosis.—In chlorosis it is the reduction of hæmoglobin with the slight numerical variation from normal of the red cells that makes for a diagnosis. The color index is very low. There is nothing abnormal about the leukocytes. Microcytes may be present, and very occasionally a normoblast. Macrocytes and megaloblasts are always absent.

Blood of chlorotics is very pale and very fluid and coagulates rapidly, hence frequency of thrombosis. There is an increase in the blood platelets.

Spleen, liver, and lymph glands as a rule normal.

Simple Primary Anæmia.—This condition is not recognized by many authors but is a convenient term under which to group anæmias which are neither chlorosis, nor pernicious anæmia and for which no assignable cause can be designated. It is a secondary anæmia without a cause. In it color index is about normal, there is no change in the leukocytes and cases go on to recovery.

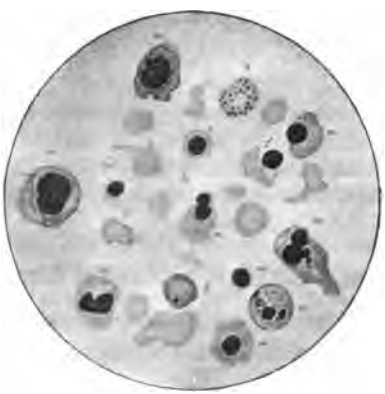


FIG. 62.—Pernicious anæmia. *M.m*, Megaloblasts; *n*, normoblast; *s*, stippling (punctate basophilia). (*Cabot.*)

Pernicious Anæmia (Addison-Biermer Anæmia).—In pernicious anæmia we obtain a very fluid, but normally colored drop of blood upon puncture. The yellow marrow of the long bones is transformed into a soft, bright red lymphoid tissue, smears from which may show great numbers of megaloblasts.

Areas of fatty degeneration are characteristic, especially the tiger-lily spots in the heart muscle. Iron-containing pigment (hemosiderin) is found in the liver, spleen, and kidneys. Areas of degeneration in the spinal cord may account for nervous symptoms. The red cells frequently fall below 2,000,000 with patients going about. Cases have been reported with counts under 200,000. The color index is high. Megaloblasts are the most characteristic qualitative change in the red cells. Delayed coagulation and decrease in blood platelets are often noted. With the high color index there is leukopenia. In my opinion a marked anisocytosis is more a feature of the disease than the presence of megaloblasts. Urobilinuria is marked.

Megaloblastic crises may at certain times show enormous numbers of megaloblasts. When the circulation is suddenly flooded with large numbers of nucleated reds such regenerative manifestation is called a "blood crisis." Cases often present remissions in which no megaloblasts can be found. In such cases the presence of many macrocytes should prevent an examiner's reporting against a pernicious anæmia previously diagnosed.

Poikilocytosis, polychromatophilia, and stippling are also features of the disease. Normoblasts are far less frequent than megaloblasts and there is usually a moderate lymphocyte increase and polynuclear decrease. Myelocytes may be present, but their precursors, the myeloblasts, are probably more frequently met with.

The onset of the disease is insidious, the patient developing a lemon-yellow color and weakness. Many cases fail to show any signs of emaciation. There are often accessions of alimentary tract disorders and anorexia is usually marked.

Cases of pernicious anæmia show remissions during which the patient is apparently on the road to recovery. Such improvements are only temporary. The remissions may last from two months to possibly three or four years. Especially in the anæmia of *Dibothriocephalus latus* do we have a picture of pernicious anæmia. It is supposed to be due to a toxin present in the heads of these tape-worms.

In pernicious anæmia there is usually an absence of free HCl, which causes it often to be confused with gastric cancer. Cases of chronic nephritis are also often much like pernicious anæmia but most difficult of differentiation are affections of the spinal cord, such as tabes, etc., the neurological manifestations of pernicious anæmia causing the confusion.

Blood changes more or less like those of pernicious anæmia have at times been noted in children with tuberculosis of bovine nature. The human strain of T. B. does not seem to produce such changes.

An acute disease showing a rapidly developing anæmia of the pernicious anæmia type is Oroya fever in which the bone marrow seems especially involved.

Secondary Anæmias

These are the anæmias which can be definitely traced to some disease not of the hæmopoietic system.

There are two main groups—those following hæmorrhage and those secondary to various diseases.

Acute Posthæmorrhagic Anæmias.—If the hæmorrhage is sudden and great, the resulting condition is one of oligochromæmia—chlorotic in type. Normoblasts are usually found after the third day. Blood platelets are increased and coagulation rate shortened.

In the regeneration the plasma restoration comes first, then the red cells and finally the hæmoglobin. An increase in number of polymorphonuclears is always noted with an occasional myelocyte.

The low Hb. percentage is apt to continue for several weeks.

It is a question whether prolonged operation or those requiring narcosis are justified where the reduction in Hb. is under 40%. (According to Miculicz, 30% is the minimum.)

Chronic Posthæmorrhagic Anæmias.—Where the loss of blood is gradual, as in gastric cancer or severe hæmorrhoids the picture may more nearly approach that of pernicious anæmia. Secondary anæmias usually show a moderate leukocytosis. In chronic nephritis and prolonged suppurative conditions normoblasts and macrocytes are rare—moderate poikilocytosis with the presence of many microcytes being the rule.

In fatal anæmia from chronic acetanilide poisoning high color index, macrocytes and megaloblasts have been noted.

Secondary Anæmias from Disease.—In some secondary anæmias, as in syphilis, carcinoma, and tuberculosis, we have a chlorotic color index (chloro-anæmias).

In secondary anæmias polychromatophilia, poikilocytosis, and punctate basophilia (stippling) may be present. This latter is very marked in lead poisoning, but in certain cases of malarial cachexia it may be equally prominent. The only form of nucleated red cell seen is the normoblast, in very small numbers, or it may not be present.

Megaloblasts are practically never seen, except in some of the very severe parasitic anæmias, as the broad Russian tape-worm infection. The red cells generally number between 2,000,000 and 4,000,000, thus differentiating chlorosis. The leukocytes are frequently increased to 15,000. In the anæmia of splenic anæmia there is a marked leukopenia. In anæmias from malignant tumors the color index is usually of the chlorotic type—the hæmoglobin content of the red cells being more affected than the number. Normoblasts are usually present, and this finding may differentiate gastric cancer from ulcer. In bone marrow metastases megaloblasts may be expected. Myelocytes and so-called tumor cells (large cells with faintly

staining vacuolated nuclei and but little cytoplasm) may also be found. As a rule, there is a moderate leukocytosis in malignant disease. Eosinophiles may be largely increased in sarcoma.

The Leukæmias

It is in the leukæmias that we have the greatest increase in the number of white cells. These cases show more or less anæmia, but we may have cases of myelogenous leukæmia showing 250,000 leukocytes per cubic millimeter without particular change in the red cells. The more marked the red-cell change the more severe the condition.

There are two well-defined types of leukæmia, the lymphatic and the splenomyelogenous. It must be borne in mind, however, that while a greater change in the lymphatic glands may produce the lymphatic type, yet even in such cases we expect to find alteration in bone marrow and spleen; that is, there is a general involvement of the hæmopoietic system in all leukæmias, the activity being most marked in spleen and bone marrow in certain cases and in lymphatic glands in others.

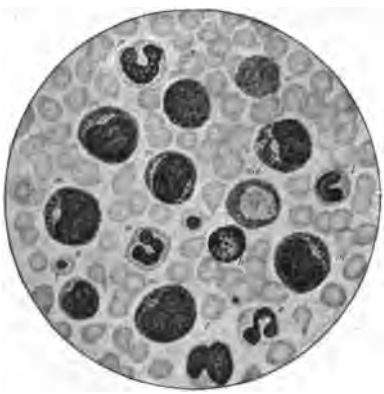


Fig. 63.—Myelogenous leukæmia. *m*, Myelocyte; *p*, polymorphonuclear; *b*, mast cell; *n*, normoblast. (*Cabot.*)

Myelogenous leukæmia is a very rare disease, about five times as rare as pernicious anæmia. Lymphoid leukæmia is still more rare.

Splenomyelogenous Leukæmia (Myeloid Leukæmia).—The differentiation of the blood picture of this disease from leukocytosis does not depend on the number of leukocytes, but on the presence and large proportion of myelocytes.

We expect both neutrophilic and eosinophilic myelocytes in myeloid leukæmia—the proportion of these varies, but, as a rule, the neutrophilic one is the common one. The blood in advanced cases is milky and shows a most marked buffy coat. The

marrow is largely replaced by a yellow pyoid material. The spleen may weigh 10 pounds.

Chronic myeloid leukæmia is insidious in onset, the first points to attract attention being the greatly enlarged spleen, pallor and prostration. Some cases have fever and night sweats thus suggesting tuberculosis.

The leukocyte count is on the average from 200,000 to 500,000. Cases are reported of more than 1,000,000 white cells. The neutrophilic myelocytes make up about 30 to 40% of these and, about equal in number, are found the polymorphonuclears, while the percentage of the lymphocytes is decreased (2 to 5%) and normal eosinophiles, eosinophilic myelocytes, and large mononuclears make up the remaining percentages. Myeloblasts may be present as well as myelocytes and in exacerbations of the disease there may be many myeloblasts. We usually have great numbers of normoblasts. Megaloblasts may rarely be found. The red count is usually about 2,500,000 and the color index low. There is an *acute myeloid leukæmia* which runs a rapidly fatal course. The characteristic cell is the myeloblast. It is rare and is often confused with acute lymphatic leukæmia.

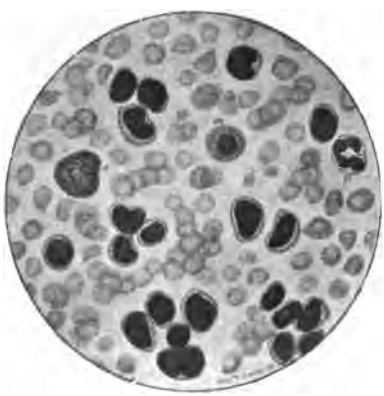


FIG. 64.—Lymphatic leukæmia. *p*, Polymorphonuclear; *m*, megaloblast; *e*, eosinophile. Twenty-one lymphocytes in this field. (*Cabot.*)

Lymphatic Leukæmia.—In this we have glandular enlargements, but not such large masses as in Hodgkin's disease. The red cells are usually reduced about one-half and the color index is a little below normal. Normoblasts are rarely found. Myelocytes, as a rule, are absent, but may amount to 5% of the leukocytes. The predominating leukocyte (75 to 98%) is the small lymphocyte. In acute lymphatic leukæmia the large lymphocytes and Rieder cells are the diagnostic ones.

These however are pathological and differ from the large lymphocyte in not having typical azur granules and the nucleus stains poorly and is often indented. The leukocyte count in chronic lymphatic leukæmia is never so great as in myeloid leukæmia, rarely exceeding 125,000.

Chronic lymphatic leukæmia has an insidious onset with increasing pallor and weakness. The glands in general tend to enlarge, most markedly, however, in the cervical region. This is one of the easiest of diseases to diagnose by a blood smear.

In *acute lymphatic leukæmia* there is a rapid course with fever and tendency to hæmorrhages. The tonsils are often swollen and accentuate the usual stomatitis. At first the white count is about normal but in a week or so may run up to 150,000.

Pseudoleukæmia.—Hodgkin's disease is usually considered as a disease with marked glandular enlargements, but with a negative blood picture, or at any rate only a moderate leukocytosis. Blood platelets are increased.

The glands in this condition do not soften and on section show diffuse hyperplasia of cells of the endothelial type. Eosinophiles are also abundant in the sections. Connective-tissue increase and small necrotic areas are also features.

The red cells are usually above 3,000,000. It has been considered that an increased percentage of transitionals (10 to 15%), should a leukopenia coexist, is characteristic.

Hodgkin's disease has an insidious onset, the enlargement of the glands of one side of the neck usually first attracting attention. The first glands to enlarge, however, may be internal ones. A relapsing type of fever often is present.

Undoubtedly the view that so-called lymphosarcomata, lymphatic leukæmia, and Hodgkin's disease merge into one another and that they represent a malignant cell formation in the hæmopoietic system is the conservative one to take.

A certain proportion of cases of Hodgkin's disease, however, show endothelial cell proliferation and a chronic fibroid change.

In **Kundrat's lymphosarcoma** we have a neutrophile leukocytosis and a diminution of the lymphocytes. The spleen and liver are rarely involved.

Another condition with swelling of the lymphatic glands, which do not however fuse, is the so-called granulomatosis.

In this we have a polymorphonuclear leukocytosis of from 20,000 to 50,000 with an increase in the percentage of eosinophiles. The lymphocytes are absolutely and relatively decreased. In granulomatosis there is no tendency to hæmorrhage.

Splenomegaly.—The best known anæmia associated with splenic enlargement is Banti's disease.

Banti's disease has a very low color index and leukopenia. In this the primary affection is of the spleen which becomes greatly enlarged. In the second stage we have a moderate anæmia of chlorotic type associated with slight weakness and dyspnœa. The accompanying cirrhosis of the liver with its symptoms of ascites, etc., marks the third stage. Splenectomy often cures the disease. The leukopenia is one showing not only a diminution of polymorphonuclear percentage but of cells of the lymphocyte type as well.

There is a considerable increase in the large mononuclear percentage. Nucleated reds and myelocytes are invariably absent. It must be remembered that we have a group of cases showing splenomegaly which are syphilitic in origin and which, as a rule, give a positive Wassermann. Clinically or hæmatologically they resemble true Banti's disease but pathologically the spleen shows a fibrosis instead of the marked increase in lymphatic tissue characteristic of Banti's disease.

In the tropical splenomegaly or **kala-azar** we have a marked leukopenia with a marked reduction in the percentage of polymorphonuclears. The **Gaucher type** of splenic anæmia does not show as pronounced and early an anæmia as in Banti's type. It is characterized by a bronze pigmentation of face and extremities and shows a family incidence. There are no constant blood changes.

In **hæmolytic jaundice** we have, in addition to the splenomegaly, jaundice and a tendency to epistaxis and purpura. It is a familial disease. The red cells show marked fragility, at times hæmolyzing in 0.7% while normal cells withstand a 0.4% salt solution.

The blood shows a marked anisocytosis with many microcytes. Normoblasts are generally present. The color index is about 1.

Certain conditions which partly resemble myelogenous leukæmia and partly pernicious anæmia are designated **leukanæmia.** Some consider this to belong to the group of diseases in which the multiple myeloma is placed.

In **splenomegalic polycythæmia** we have a red count of from 9,000,000 to 10,000,000. The Hb. percentage may be 200. There is also a leukocytosis up to 50,000. Patients are cyanosed and have a very large spleen.

Splenic anæmia of infancy usually occurs between the ages of twelve and twenty-four months. The spleen is notably enlarged and in many cases the liver is equally so. The red cells are not greatly diminished in number, 2,500,000 to 3,000,000 being usual findings. Nucleated reds are abundant. While a leukocytosis of 30,000 to 50,000 is often present it is markedly less than that of splenomyelogenous leukæmia and the increase in white cells is more of those of lymphocyte type.

The color index is very low.

Another splenomegaly of children, clinically resembling kala-azar, is caused by *Leishmania infantum.* This shows a leukopenia.

PART III
ANIMAL PARASITOLOGY

CHAPTER XV

GENERAL CONSIDERATIONS OF CLASSIFICATION AND METHODS

ANIMALS that are in all respects alike we term a species. Of course the male and female of a species may be very unlike, but as a result of mating they produce young having characteristics similar to the parents. Now, if, as in the case of the mosquitoes, certain ones of which cause yellow fever, we find some with straight silvery lines and others uniformly showing crescentic silvery bands about thorax, yet resembling each other closely in the respect of being dark, brilliantly marked mosquitoes, we should consider them as being separate *species* with a certain relationship to which the term *genus* is applied.

The term "genus" is of wider application than the word "species." Thus animals which agree in the main characteristics of size, proportion of parts, and general structure are placed in the same genus.

In naming a species we always first write the name of the genus which has a Greek or Latin name, commencing with a capital, and follow with the specific name, which latter commences with a small letter. Thus we designate the dark silver-marked mosquitoes as belonging to the genus *Stegomyia;* those showing the characteristics of curved silver bands and two central parallel lines (lyre pattern) on dorsal surface of thorax we designate as *Stegomyia calopus;* the species with only the straight silver lines we call *Stegomyia scutellaris.*

The specific name may be a noun in the genitive. If an adjective it must agree in gender with the generic name.

It is permissible to have a masculine noun as a specific name with a feminine generic name.

If the specific name is a modern patronymic we add i in the case of a man or æ for a woman to the exact and complete name of the person.

Again, certain genera show resemblances which enable us to make broader groupings to which we apply the term *subfamily*. Thus the genus *Stegomyia* and the genus *Culex* have the similar characteristics of palpi in the female being shorter than the straight proboscis; we therefore classify all species of *Stegomyia* and all species of *Culex* under the designation Culicinæ. The name of a subfamily ends in "inæ." Now, again, certain insects are different from others in having scales on the wings. We find that not only do the Culicinæ have such characteristics, but the same is observed with the Anophelinæ and other similar scale-wing insects. All of these we term a *family* and we speak of the Culicidæ, meaning the family of mosquitoes. The name of a family ends in "idæ." Many families are not subdivided into subfamilies, but are directly separated into genera. Again, a genus may have only a single species.

At times a family may be raised to *superfamily* rank—the subfamilies then becoming families. Thus the families Ixodidæ and Argasidæ belong to the superfamily Ixodoidea. The termination for a superfamily is "oidea."

When there are a number of families agreeing closely in some striking characteristic, we group them together into an *order;* thus, the family of mosquitoes closely resembling many other families of insects in possessing a pair of well-developed wings are grouped in the order Diptera; all of which resemble certain other animals in the possession of a distinct head, thorax and abdomen with three pairs of legs projecting from the thorax. This collection of animals we call a *class;* thus, we speak of the class Insecta. It will be observed that the insects have no internal skeleton, but instead a chitinous cuticle, the exoskeleton. Spiders, ticks, etc., resemble them in this respect, and we now apply to all such animals the wider designation, *branch* or *phylum* Arthropoda.

Inasmuch as the animal kingdom is divided into the branches Protozoa, Porifera, Cœlenterata, Echinodermata, Vermes, Arthropoda, Mollusca and Chordata, we see that the branch is the largest grouping we employ. To descend in the scale we have belonging to the branch, the classes; to the class, the orders; to the order, the families; to the family, the subfamilies; to the subfamily, the genera; to the genus, the species. Occasionally a species is further divided into subspecies.

By a *type species* we understand the species of a genus always referred to as representing the genus.

While other species of a genus may for good reason be transferred to another genus the type species is permanently in the genus. Many favor alliteration for type species, as *Heterophyes heterophyes*. When a species is transferred to a new genus the specific name goes with it.

The male animal is designated by the sign of Mars (♂), the female by that of Venus (♀).

CLASSIFICATION OF PHYLA OF IMPORTANCE IN ANIMAL PARASITOLOGY

(According to Stiles)

1. Unicellular animals, as the parasites of malaria.....Protozoa.
   Pluricellular animals; metazoa..... 2
2. Body more or less flattened dorsoventrally..... 4
   Body ordinarily round in transverse section..... 3
3. Body never annulated, never provided with legs; no jaws present..... 5
   Body annulated, or at least provided with mouth parts; usually breathe through a tracheal system; adults with jointed legs..... 7
4. Intestine, but no anus, present; one or two suckers present; body not segmented; parasitic in liver, lungs, blood, intestine; occasionally elsewhere; flukes..... Trematoda.
   Intestine absent; two or four suckers on head; body of adults segmented; tissue usually contains calcareous corpuscles; adults (tape-worms) parasitic in intestine; larvæ (bladder worms) parasitic elsewhere.....Cestoda.
   Intestine and anus present; ventral sucker on posterior end; body annulated like an earthworm; parasitic in upper air-passages, or externally, leeches, blood-suckers.....Hirudinei.
5. Intestine absent; armed rostellum present; very rare in man, in intestine, thorn-headed worms.....Acanthocephali.
   Intestine present; no armed rostellum..... 6
6. Intestine rudimentary in adult; rare, accidental parasites in intestine of man, hair snakes or horse-hair worms.....Gordiacea.
   Intestine present; parasitic in intestine, muscles, lymphatics, etc., very common and important; roundworms.....Nematoda.
7. Six legs present in adult; wings present in most species; larvæ annulated much like an earthworm; breathe through tracheæ; adults ectoparasites; occasionally larva is parasitic under skin, or in wounds, or an accidental parasite in the intestine; insects.....Insecta.
   Eight legs present in adult, six legs in larva; head and abdomen coalesced; ectoparasites; some burrow under the skin or live in the hair follicles; acarines.....Acarina.
   Four claws around the mouth; larva encysted in various organs; adult occasionally parasitic in nasal passages; tongueworms.....Linguatulidæ.
   Numerous legs present; occasionally accidental parasites in nasal passages or intestine, thousand leggers.....Myriapoda.

There are certain terms employed in animal parasitology which it is necessary to understand. Among these we shall refer to the following:

1. **True Parasitism.**—By this is understood the condition where the parasite does harm to the host, deriving all the benefit of the association. A good example of this would be the hookworm infecting man or animals.

2. **Mutualism.**—In such an association there is mutual benefit to each party of the association. An instance of this would be the presence of colon bacilli in the in-

testines. The bacillus is furnished a suitable habitat and in return protects its host against strictly pathogenic bacteria.

Another example would be the oyster crab found inside the oyster shell.

3. **Commensalism.**—Here there is benefit to the parasite, but no injury to the host. An example of this kind would be furnished in the case of the *Trichomonas vaginalis* which lives in the vaginal mucus, but so far as known, does no injury to the host.

If the *Entamœba coli* be nonpathogenic this would be another example.

4. **Nomenclature.**—When the thousands of different species, genera, etc., of animals are considered, it will be readily perceived that, unless some system existed for their designation, indescribable confusion would prevail. To avoid this, the International Code, based on the rules of Linnæus (tenth edition of Systema naturæ, 1758, is basis of binary zoological nomenclature), requires Latin or Latinized names.

In printed matter the zoological name should be in italics, that of the family in Roman type. The name of the author of a specific name is written immediately after the name without punctuation and may be followed by the year of publication set off by a comma, thus: *Ascaris lumbricoides* Linnæus, 1758. Should the name of the author appear in parentheses it indicates that he proposed the specific name but placed the species in another genus than that in which it now appears, and the name of the author responsible for placing the species in the present genus may be written after the name of the original author of the species; for example, *Davainea madagascariensis* (Davaine, 1869) Blanchard, 1891, tells us that Davaine proposed the specific name *madagascariensis* in 1869 but placed it in some other genus and that Blanchard in 1891 transferred it to the genus *Davainea*. There are certain rules governing the naming of animals. Of these, the law of priority provides that the oldest published name, under the code, of any genus or species is its proper zoological name. The history of the naming of the organism of syphilis illustrates this well. Schaudinn gave this organism in 1905 the name of *Spirochæta pallida*. Ehrenburg, in 1838, had used the name *Spirochæta* for animals of a different character, so that this designation of the genus was not permissible under the code. Villemin, a little later, proposed the generic name *Spironema*. This term, however, was found to have been used in 1864 by Meek for a genus of molluscs and by Klebs in 1892 for a genus of flagellates. Consequently, being a homonym, it was not available.

(A generic name can be applied to only one animal genus and if a similar name is subsequently given another genus it is a homonym and is to be rejected.)

On December 2, 1905 Stiles and Pfender then proposed the name *Microspironema*, but as Schaudinn published on Oct. 26, 1905 the designation *Treponema*, the name *Treponema pallidum* had to be accepted as the proper zoological name for the organism of syphilis.

Of unusual interest is the question of the name of the old-world hookworm. Dubini, in 1843, named a nematode found by him in man *Agchylostoma*. By the law of priority this spelling would have been the correct one had he not stated in a footnote that the generic name was derived from two Greek words αγχύλοσ and

στόμα. Having indicated the origin of the name it became subject to the rules for correct transliteration, which is *Ancylostoma*.

In case of larva and adult or male and female, formerly considered different animals but subsequently found to be the same, the oldest available name becomes the name of the species.

Another point is that names are not definitions, consequently the fact of lack of appropriateness of any name is no objection to its continuation. This will appeal to anyone as a wise provision, because if a different name were substituted each time a designation more descriptive or applicable was invented it would be utterly destructive to system. When it is considered that some of our parasites have approximately fifty different designations, for the most part given by medical observers, it will be appreciated how much the zoologist has aided us in trying to eliminate all but the single proper zoological name.

It is a rule of zoological nomenclature that zoological names are independent of botanical ones so that the prior use of a generic name for a plant is not an objection to its use for an animal.

The objections so frequently heard among physicians in connection with adopting new names for old ones are not well founded. Wherever confusion has reigned, the establishment of order always results in temporary greater confusion. There is no doubt that the student taking up this subject a few years hence will have the satisfaction, thanks to the zoologist, of only having to burden his mind with one name for each parasite.

There is only one correct name for an animal and all other names are synonyms.

The principal cause of changes of names is that our conception of the relationships of animals changes.

5. **Terminology.**—This applies to appropriate designations for different organs, symptoms, etc., and is not subject to any rule other than that of good usage.

Thus the terms cirrus in the case of the male copulatory organ of flukes, spicule for the same in nematodes and penis in connection with insects would be instances of terminology.

6. **Pseudoparasitism.**—Where organisms enter the body accidentally and when such sojourn in the body of man plays no part in the life history of the organism we employ the term pseudoparasitism. For example: Fly larvæ swallowed by man and passed out in the fæces. We also use the terms temporary parasites (bedbug) and permanent parasites (liver fluke).

7. **Hosts.**—The animal in which a parasite undergoes its sexual life is called the definitive or final host, that in which it passes its larval existence the intermediary host. For example: Man is the intermediary host of the malarial parasite, the mosquito the definitive host. A single animal may, however, be both definitive

and intermediary host; thus *Trichinella* may pass its larval existence in the muscles of man and its sexual life in his intestines. With certain infections we have two intermediate hosts as in paragonomiasis where the first intermediate host is a mollusc and the second intermediate one a crab.

8. **Heredity, Congenitalism.**—Hereditary characteristics are those which were present in the ovum or spermatozoon before fertilization; congenital ones those which occur after fertilization. South African tick fever is probably an instance of heredity, the spirochætes having been found in the ovary and ova of the female tick.

9. **Heterogenesis, Parthenogenesis.**—Offspring differs from parent, but after one or more generations there is reversion to the parent form.

Strictly speaking the term heterogony applies to reproduction when a sexual generation alternates with a parthenogenetic one. Where a nonsexual generation, as by division or budding, alternates with a sexual one the process is called metagenesis. In parthenogenesis reproduction eggs develop without the occurrence of fertilization by spermatozoa.

In coccidiosis we have a sexual cycle (sporogony) alternating with a nonsexual one (schizogony). In the infection with *Strongyloides* we have a sexual cycle alternating with a parthenogenetic one. In malaria we have a sexual generation, a nonsexual one and according to Schaudinn, a parthenogenetic one, which latter accounts for malarial relapses.

10. **Homology and Analogy.**—By homology we understand the anatomical correspondence of the organ of one animal to that of another. Thus the foreleg of a quadruped and the wing of a bird are homologous organs. Analogy refers to physiological or functional agreement, thus the lungs of mammals and gills of fish, both with respiratory functions, are analogous organs. The first trace or appearance of an organ in an embryo is known as the *anlage of the organ.*

11. **Protista.**—Haeckel proposed this name for unicellular animals and plants, thus including protozoans and protophytes in a kingdom separate from the animal and vegetable kingdoms.

We have sufficient difficulty in drawing the line between an animal and vegetable organism so that to make a demarcation of a new kingdom from the two usually recognized would add to our difficulties.

12. **Phylogeny and Ontogeny.**—Phylogeny deals with the evolution of a group of animals. The phylogenetic or ancestral history of the genesis of the horse of the present day shows that it developed from an animal with 4 toes on the fore foot and three on the hind foot. Ontogeny deals with the evolution or germ-history of an individual. It deals with the development from egg to mature adult.

CHAPTER XVII

THE PROTOZOA

Classification of Protozoa

Class	Order	Genus	Species
Rhizopoda (Sarcodina) — These throw out protoplasmic projections called pseudopodia.	Gymnamœba	Entamœba	E. coli
			E. histolytica
			E. buccalis
		Leydenia	L. gemmipara
Flagellata (Mastigophora). — These move by means of undulating membranes or flagella.		Spirochæta	S. recurrentis
			S. vincenti
			S. duttoni
			S. carteri
			S. icterohæmorrhagiæ
			S. refringens
		Schizotrypanum	S. cruzi
		Treponema	T. pallidum
			T. pertenue
		Trypanosoma	T. gambiense
			T. rhodesiense
		Trichomonas	T. vaginalis
			T. intestinalis
		Tetramitus	T. mesnili
		Lamblia	L. intestinalis
		Prowazekia	P. asiatica
		Leishmania	L. donovani
			L. tropica
			L. infantum
Infusoria (Ciliata) — These have contractile vacuoles and numerous fine cilia which are shorter than flagella and have a sweeping stroke.	Heterotricha	Balantidium	B. coli
		Nyctotherus	N. faba
Sporozoa — These have no motile organs. They live parasitically in the cells or tissues of other animals. Reproduction by spores.	Coccidiaria	Eimeria	E. stiedæ
		Isospora	I. bigemina
	Hæmosporidia	Plasmodium	P. vivax
			P. malariæ
			P. falciparum
		Babesia	B. bigemina

NOTE.—Hartmann and others have grouped the Hæmosporozoa and the Hæmoflagellata in an order BINUCLEATA. The main characteristic is the possession of two differentiated nuclei, the kinetonucleus and the trophonucleus, at some developmental or transitional stage. While trypanosomes plainly show these characteristics certain others, as the malarial parasites and the Leishman-Donovan bodies, having been modified as the result of cell parasitism, do not do so. This grouping together of the blood flagellates and sporozoa under the name Binucleata has been considered by many protozoologists as possibly convenient but not resting on sufficient ground to cause organisms with similar life histories as *Plasmodium* and *Coccidium* to be separated and the former to be placed with the blood flagellates in a new grouping.

It is a question as to whether the cellular bodies in the ascitic fluid of two cases in Berlin were really protozoa and to which the name *Leydenia gemmipara* was given. A protozoon, *Chlamydophrys enchelys*, has been found in fresh human fæces. This rhizopod has an oval hyaline shell with a terminal orifice from which filiform pseudopods project. It has been considered that the protozoa classed as *Leydenia* were really abnormal *Chlamydophrys*.

THE PROTOZOA

By the term protozoa we understand a branch of animals in which a single cell is morphologically and functionally complete; it is not one of a number of cells going to make up a complex individual and dependent on such a combination as is the case with the metazoa (there is no differentiation into tissues in protozoa).

Recognizing the fact that certain protozoa have characteristics which make it impossible to draw a distinction between them and plants Haeckel has proposed the name Protista as a designation for all simple and primitive living organisms whether they be plants or animals. In such a classification we would have the kingdom of Protista as well as the animal and vegetable kingdoms. In such a grouping the bacteria would be the lower types and the fungi and protozoal organisms the higher ones.

The protozoal cells are made up of protoplasm which is divided into nucleus and cytoplasm. The cytoplasm is at times separated into an external, hyaline portion, the ectoplasm or ectosarc and an internal granular portion, the endoplasm or endosarc. The functions of the ectosarc are protective, locomotor, excretory and sensory; those of the endosarc trophic and reproductive. Protozoa may be holozoic (animal like) or holophytic (plant like), saprophytic (fungus like), or parasitic (living at the expense of some other animal or plant).

The nucleus is characterized by concentration of the so-called chromatin substance of the cell. This chromatin however is usually combined with achromatin. The usually accepted test for chromatin is the staining affinity for basic aniline dyes. This test is now known to be unsatisfactory as other substances than chromatin may stain even more intensely. When chromatin is scattered through the cytoplasm, as extranuclear aggregations, such chromatin granules are called chromidia. There are cells where the chromidia take the place of the nucleus and from which a nucleus may be formed. Chromidia may arise from nuclei and nuclei from chromidia. The

nucleus is made up of a network of linin in which achromatic reticulum is contained the nuclear sap or karyolymph. As a rule an achromatic nuclear membrane, continuous with the reticulum, separates the nucleus from the cytoplasm. In addition we have a substance which is achromatic (plastin) and which is the imbedding substance for chromatin grains. These plastin chromatin combinations are called karyosomes. The nucleoli are probably pure plastin. Plastin is to be regarded as a secretion or modification of chromatin made to serve as a matrix for the chromatin. Chromatin may be concentrated in a single mass so that the nuclear space looks like a vesicle with a central chromatin mass (vesicular nucleus) or numerous chromatin grains may be scattered through the nuclear space (granular nucleus). The centrosome, which presides over cell division, is usually located just outside the nucleus. In some protozoa however the centrosome is within the nucleus and is often seen inside of a karyosome and is then called a centriole. The centrosome may also function over kinetic activities (flagellar motion) and is then termed blepharoplast.

When appearing as a small granule at the base of the flagellar apparatus it is called the basal granule. When there are extensions from it to the nucleus we have rhizoplasts.

Certain protozoa, as trypanosomes, show a differentiation of nuclei, the larger trophonucleus governing the functions of general metabolism and the smaller kinetonucleus directing the motor activities. Infusoria have a larger macronucleus which contains vegetative chromatin and a smaller micronucleus which contains reserve reproductive chromatin.

1. Reproduction of protozoa may be by fission, when the nucleus and cytoplasm divide into two by simple division.

When the nuclei divide into a number of daughter nuclei, which is followed by multiple division of the cytoplasm, we have sporulation.

2. Instead of fission we may have sexual reproduction or conjugation (zygosis). Here the nuclei of the separate sexual individuals (gametes) are termed pronuclei and the product of their fusion a synkaryon.

a. Where a single cell has division of its nucleus with subsequent fusion of these daughter nuclei to form a synkaryon the process is termed autogamy.

b. If two similar cells conjugate the term is isogamy; if dissimilar as the macrogametes and microgametes of malaria, anisogamy.

The process of sexual union is termed syngamy and is of two kinds (1) when the two gametes fuse completely or copulation and (2) when they remain separate and only exchange nuclear material or conjugation.

The structures of protozoa concerned in movement, metabolism, etc., are termed organelles. Of the former, pseudopodia, flagella, cilia and myonemes (contractile fibrils which give support to the body cell of certain protozoa) may be given and food vacuoles and contractile vacuoles of the latter. The contractile vacuole which is probably an excretory organelle is absent in almost all parasitic protozoa. It is however present in ciliates.

RHIZOPODA (SARCODINA)

In this class of protozoa the pseudopodia serve the double purpose of nutrition and locomotion. These protoplasmic extensions may be quite broad or very narrow—the lobose and the reticulose.

As a rule, the thicker the pseudopod the more rapid the movement. Some rhizopods have hard shell-like coverings which are secreted in or on the ectosarc. These skeletons have openings through which the pseudopods project. The pseudopodia may be made up only of ectoplasm or both ectoplasm and endoplasm may take part. Amœboid movement always starts in the ectoplasm. In addition to the nucleus, which the so-called chromatin-staining methods of Romanowsky brings out as reddish areas, or black with iron hæmatoxylin, we frequently observe aggregations of chromatin-staining material in the cytoplasm. These cytoplasmic chromatin bodies (chromidial bodies) are of importance in differentiating the encysted pathogenic amœba from the nonpathogenic one. This extranuclear chromatin is supposed to play a part in the more intricate divisions which such protozoa undergo. Food vacuoles and contractile vacuoles are present in many rhizopods.

Intestinal Amœbæ

There are certainly two species of intestinal amœbæ having man for a host, the one pathogenic, *Entamœba histolytica* and the other a harmless commensal, *Entamœba coli*.

Schaudinn, in 1903, described the pathogenic amœba, which he named *E. histolytica*, as follows: 1. Distinct, highly refractile and tenacious ectoplasm. He considered this tough external portion of the cytoplasm as the explanation of the ability of the pathogenic amœba to bore its way into the intestinal submucosa. 2. Eccentric nucleus which was indistinct by reason of little chromatin. 3. Reproduction by peripheral budding in which small aggregations of chromatin reached the periphery of the cytoplasm and, enclosed in a resistant capsule, broke off from the parent amœba and constituted the infecting stage.

For the nonpathogenic *E. coli* he noted, 1. No distinction between a granular endoplasm and refractile ectoplasm. 2. Centrally placed and sharply outlined nucleus, rich in chromatin and 3. Encystment with the formation of eight nuclei, which nuclei or amœbulæ form the infecting stage.

The pseudopodia of *E. histolytica* are actively projected as long finger-like processes which show the ectoplasm quite distinctly, while the pseudopodia of *E. coli*, are lobose and sluggishly projected and show a uniformly opaque grayish color.

In 1907 Viereck and later Hartmann recognized a pathogenic amœba with four nuclei in its encysted form, to which was given the name *E. tetragena*.

All authorities now consider that Schaudinn made an error in observation as to the existence of peripheral budding for *E. histolytica*, so that we recognize but two types of encystment, one with a larger cyst and thicker cyst wall, with eight nuclei and an absence of chromidial bodies—*E. coli* and the other, smaller, with a thin cyst wall, four nuclei and chromidial bodies in the encysted stage, the pathogenic amœba, *E. histolytica*. Synonym. *E. tetragena*.

In the vegetative stage the human amœbæ are best differentiated by the nuclear structure. In *E. coli* the nucleus is vesicular with a thick nuclear membrane and the chromatin chiefly deposited on the undersurface of the nuclear membrane. In

hæmatoxylin-stained specimens this chromatin often seems deposited in quadrant aggregations.

For the pathogenic amœba we recognize a *histolytica* type of nucleus, which is found in dysenteric stools, and a *tetragena* type, which is found in diarrhœal or more or less normal stools.

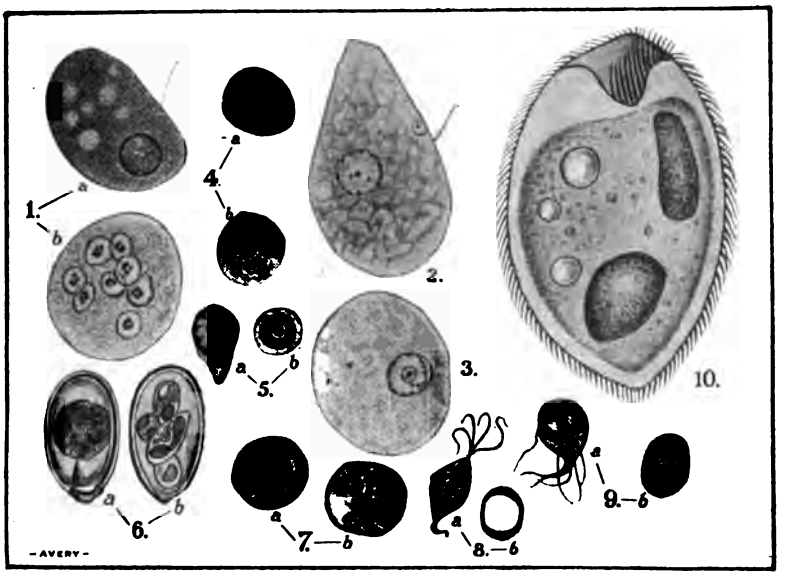


FIG. 65.—Important pathogenic protozoa of the intestinal tract. (1a) Motile *E. coli*. Note large amount and peripheral arrangement of chromatin in nucleus. (1b) Encysted *E. coli*. Note larger size than *E. histolytica* cyst, 8 ring form nuclei and absence of chromidial bodies. (2) Motile *E. histolytica* from acute dysenteric stool. Note histolytica nucleus with scanty chromatin. (3) Tetragena type of *E. histolytica* from case of chronic dysentery. Note greater amount of chromatin and central karyosome with centriole. (4a) Preëncysted *E. histolytica* from carrier. Note small size and heavy peripheral ring of chromatin in nucleus making this feature of chromatin in nucleus similar to the larger *E. coli*. (4b) Encysted *E. histolytica* from dysentery convalescent. Note small size, 4 ring nuclei and a dark chromatin staining mass, "chromidial body." (5a and 5b) Motile and encysted cultural amœbæ from Manila water supply. (6a and 6b) Oocyst and sporozoite production in 4 spores of *Eimeria stiedæ*. (7a and 7b) Oocyst with 2 sporoblasts and oocyst with 2 spores containing 4 sporozoites of *Isopora bigemina*. (8a and 8b) Vegetative and encysted *Trichomonas intestinalis*. (9a and 9b) Vegetative and encysted *Lamblia intestinalis*. (10) *Balantidium coli*. (*Illustrations of amœbæ from Walker —others from Doflein.*)

About the time that the acute dysenteric symptoms are subsiding we often find small types of the pathogenic amœbæ. These are designated *minuta* types and have been considered by some to represent a distinct species, *E. minuta*.

The histolytica nucleus has a thin nuclear membrane and is poor in chromatin while the tetragena nucleus has more chromatin, showing radial projections from the inner surface of the nuclear membrane, and a loose central karyosome, which contains a central chromatin dot or centriole, with a clear halo surrounding it.

The preëncysted *E. histolytica* has a nucleus closely resembling that of *E. coli*. The smaller size and chromidial bodies are differentiating.

Animal experimentation upon kittens with *E. coli* by Schaudinn, Craig and Wenyon have been unsuccessful as to production of dysenteric manifestations. On the other hand all of these experimenters produced typical lesions and dysenteric manifestations in kittens injected rectally or fed with material containing pathogenic amœbæ.

Gastric juice tends to destroy vegetative amœbæ.

Darling has been so successful in his experimental work with kittens that he compares the colon of a kitten to a test-tube and suggests the procedure of rectal injections of material containing amœbæ as a means of differentiating the two human amœbæ.

On the other hand Walker was unable to infect kittens and monkeys with material containing pathogenic amœbæ and he makes the statement that such failures would indicate the greater susceptibility of man to infection, as he was able to infect 17 out of 20 men with one feeding of such material.

Wenyon produced a liver abscess in one of his experiments. In man the dislodgment of amœbæ containing material from amœbic intestinal ulcerations and the plugging of the portal capillaries by such emboli gives us the starting-point of a liver abscess. The exciting cause is *Entamœba histolytica* which in the liver continues the same production of a gelatinous necrosis as is carried on in the submucosa of the large intestine or appendix

Sellards and Baetjer note that inoculation of kittens per rectum or by feeding dysenteric stools rich in amœbæ has resulted in infection in about 50% of experiments.

By inoculating the material directly into the cæcum they were able to infect every one of their kittens. They were also able to propagate a strain of amœbæ through a series of animals for several months.

The intracæcal inoculations yielded positive results in diagnosis of human amœbiasis when the clinical manifestations were obscure and the amœbæ in the discharges so few and atypical as to make such an examination unsatisfactory.

Wenyon has reported the rather frequent occurrence in Egypt of a small amœba, *E. nana.*

The vegetative form is quite small (5 to 10 μ). They move sluggishly and have blunt pseudopods. The cytoplasm may either be vacuolated or homogeneous. The nucleus shows a large irregularly shaped karyosome. When encysted this amœba resembles small cysts of *E. histolytica* but these cysts are typically oval,

like those of *Lamblia*. The nucleus shows a chromatin mass at one side of the nuclear membrane. *E. nana* is considered to be pathogenic. It may be confused with *Amœba limax*, but these amœbæ can be cultured while *E. nana* resists cultivation.

Human Experiments.—Recently Walker and Sellards have published a most important paper.

The experiments were made in men who had been under observation for years at Bilibid Prison, whose food was cooked and the water they drank distilled. Moreover, there were complete records of examination for intestinal parasites, including entamœbæ. They were under complete control and the existence or possibility of natural infection with amœbæ was reduced to a minimum. All the men fed pathogenic amœbæ were volunteers and each signed, in his native dialect, an agreement to the conditions of the experiment.

The first series of experiments was with cultural amœbæ, in order to refute statements that amœbæ cultivated from water or other nonparasitic sources, as well as from dysenteric stools, are capable of living in man parasitically or of producing dysenteric symptoms. Twenty feeding experiments on ten men were made by Walker and Sellards with cultures of amœbæ without the development in a single instance of dysentery or the finding of such amœbæ in the stools upon microscopical examination. In 13 cases they recovered the amœbæ in cultures from the fæces from the first to the sixth day, but never afterwards. They stated definitely that cultural amœbæ are nonpathogenic.

Th next experiments were with *Entamœba coli*. In the 20 cases fed with material containing *Entamœba coli* there was a uniform failure to recover them culturally and in no instance was dysentery produced. Seventeen became parasitized as the result of a single feeding in from one to eleven days, the entamœbæ being found in the stools and persisting in their appearance in the stools for extended periods. They concluded that *Entamœba coli* is an obligate parasite, nonpathogenic, and cannot be cultured.

The third series of 20 feedings, carried on by Walker alone, was with *Entamœba histolytica*. The material was mixed with powdered starch or magnesium oxide and given in gelatin capsules. In these experiments they obtained tetragena cysts in the stools of men fed only motile *Entamœba histolytica*, and motile *Entamœba histolytica* in the stools of men who were fed only tetragena cysts and, finally, an alternation of motile *E. histolytica* and tetragena cysts in the stools of a man having a recurrent attack of amœbic dysentery.

Results.—Seventeen of the men became parasitized after the first feeding; 1 required three feedings, and 2, who did not become parasitized at the first feeding, were held as controls. The average time for parasitization was nine days. Only 4 of the 18 parasitized men developed dysentery, which came on after twenty, fifty-seven, eighty-seven, and ninety-five days, respectively, after the ingestion of the infecting material.

In 4 cases fed with material from acute dysenteric stools or from amœbæ-containing pus from liver abscess, and containing motile amœbæ, there was no resulting dysentery, the 4 cases of experimental dysentery resulting from feeding of material from normal stools of carriers.

As regards the cases which became parasitized, but did not develop dysentery, it is suggested that the amœbæ live as commensals in the intestine of the host and only penetrate the intestinal mucosa and become tissue parasites when there occurs depression of the natural resistance of the host or as the result of some lesion of the intestine. That the pathogenic amœbæ are more than harmless commensals, however, is shown by the fact that they alone, and not the nonpathogenic *Entamœba coli*, are capable of penetrating a possibly damaged intestinal mucosa.

Epidemiology.—The old idea that water, fruit or vegetables, from which one can isolate amœbæ upon culture, are sources of infection must be abandoned, as such cultural amœbæ are known to have no pathogenic relation to man.

The chief factor in the spread of amœbic dysentery would seem to be the encysted amœbæ in the stools of convalescents or healthy carriers rather than the motile ones in dysenteric stools. This probably explains the endemic rather than epidemic characteristics of the spread of amœbic dysentery because if the innumerable vegetative amœbæ in dysenteric stools were equally operative with the more sparsely eliminated cysts there would be epidemics of amœbic dysentery similar to those of bacillary dysentery.

Our present view is that the carrier is the chief factor in the spread of amœbic dysentery and when such an individual has to do with the preparation of food he becomes a particular source of danger.

Vegetative amœbæ undergo disintegration in a short time after the stool is passed, so that they are probably rarely concerned in amœbic infections but the resisting cysts may be washed from a dried stool into a water supply or even be transported in dust to lodge on unprotected foodstuffs.

Flies may possibly act as transmitting agents. As bearing on the probable importance of such flies as *Musca domestica* and *Fannia canalicularis* in transmitting amœbic infections may be noted the findings of Wenyon that the fæces of such flies, as well as *Lucilia* and *Calliphora*, after feeding on cyst-containing human fæces, teem with such cysts.

We can as a rule differentiate bacillary from amœbic dysentery by the more sudden and acute onset of the former together with fever and other evidences of toxæmia.

Again the number of stools in bacillary dysentery is usually greater and the amount of each stool less in quantity. The stool of bacillary dysentery is of a milky whiteness from the large number of pus cells, while that of amœbic dysentery is more viscid and tinged with disintegrated blood giving it a grayish green or brown color. The mucopurulent mass in bacillary dysentery may be flecked or streaked with blood. The therapeutic results following emetine injections are of value in diagnosis. Gangrenous types of dysentery are similar whether due to bacillary or

amœbic infection. Chronic dysentery of bacillary origin is much like amœbic dysentery clinically.

Laboratory Diagnosis.—The mucoid mass of amœbic dysentery is often brownish. The pathogenic amœba shows active finger-like processes and in acute attacks often show contained red cells. In the fresh specimen of the milky mucopurulent mass of bacillary dysentery one observes large numbers of pus cells and particularly very large phagocytic cells which greatly resemble amœbæ. Upon staining with Gram's stain one may find numerous Gram negative bacilli in the cytoplasm of the cell.

The large cells which resemble amœbæ are often vacuolated, thus intensifying the similarity. They are nonmotile, however, and do not show the small ring nucleus which is so characteristic of the vegetative human amœbæ. The nucleus of the confusing cells is also larger, approximating one-fourth the size of the cell.

For bringing out the nuclear characteristics of human amœbæ Walker recommends fixation of thin moist smears in Giemsa's sublimate alcohol (absolute alcohol 1 part, sat. aq. sol. bichloride 2 parts) for ten to fifteen minutes. These smears are then well washed with water and stained with alum hæmatoxylin for five minutes. The nuclear characteristics have been noted previously.

An excellent iron hæmatoxylin method is that of Rosenbusch:

Rapidly smear out with a toothpick a small particle of fæces or other material containing protozoa and, while still moist, fix by Giemsa's method and, after getting rid of the mercury with iodine followed by 95% alcohol, treat smears with a 3.5% solution of iron-alum in distilled water for one-half hour or over night, then wash thoroughly in distilled water.

Then stain from five to twenty minutes in the following hæmatoxylin stain: (1) 1% solution of hæmatoxylin in 95% alcohol. It takes at least ten days to ripen. (2) A saturated solution of lithium carbonate. Add to 10 c.c. of the hæmatoxylin solution 5 to 6 drops of the lithium carbonate one. Next wash well and differentiate with about 1% solution of the iron alum. Again wash in water pass through alcohols to xylol and mount in balsam.

With vegetative amœbæ I have obtained beautiful results with vital staining which can best be done by tinging the fæces emulsion with a 1% aqueous solution of neutral red. Cutler and Williamson state that *E. histolytica* alone will take up the rose pink color of neutral red and this staining is taken up solely by the endoplasm. They state that *E. coli* will not take the stain. They note that the "minuta" type takes the stain as well as the "tetragena" type.

I have also had good results by emulsifying the fæces in a drop of 1 or 2% formalin and then adding a drop of 2% acetic acid. The mixture is then tinged with either neutrayl red or methl green.

For distinguishing the encysted form of *Entamœba coli* one can obtain excellent

results by emulsifying the fæces in Gram's iodine solution. Owing to the glycogenic reaction given by *E. coli*, the round amœba, with its 8 nuclei stands out very distinctly.

For diagnosing the 4-nucleated cyst of the pathogenic amœba one gets better results with hæmatoxylin as this brings out not only the 4 nuclei but the chromidial bodies as well.

It was formerly customary to recommend the administration of salts prior to examining for amœbæ. Walker warns that such a procedure gives us amœbæ which are difficult to differentiate, the nuclear characteristics of *E. coli* and the tetragena nucleus of *E. histolytica* being much alike as they both contain much chromatin. In a dysenteric stool the histolytica type of nucleus, containing but little chromatin, does not resemble the nucleus of *E. coli*.

He prefers the examination of formed stools obtained without a purgative.

Walker also notes the advantages of examining a specimen with a ⅔ inch objective as encysted amœbæ are easily picked up. In opposition to the usual recommendation of text-books to report only on motile amœbæ, he recommends the making of a differential diagnosis on nonmotile encysted forms. This however is now generally accepted by experienced workers as true.

The preëncysted *E. histolytica* has a nucleus much resembling that of *E. coli*. The presence of the same chromidial bodies one notes in the cysts is an aid in recognizing this stage. The 4 nuclei of the cysts are much smaller than the nucleus of the preëncysted or vegetative stage.

As differentiating the two entamœbæ Walker gives the following table:

MOTILE STAGE

A. Entamœba histolytica	B. Entamœba coli
1. Appearance hyaline.	1. Appearance porcelaneous.
2. Refractiveness more feeble.	2. Refractiveness more pronounced.
3. Movements active in the fresh stool.	3. Movements sluggish.
4. Nucleus more or less indistinct.	4. Nucleus distinct.
5. Chromatin of nucleus scanty.	5. Chromatin of nucleus abundant.

ENCYSTED STAGE

A. Entamœba histolytica	B. Entamœba coli
1. Cyst smaller.	1. Cyst larger.
2. Cyst less refractive.	2. Cyst more refractive.
3. Cyst usually contains elongated refractive bodies known as "chromidial bodies."	3. Cysts do not contain "chromidial bodies."
4. Nuclei never more than 4.	4. Nuclei 8, occasionally more.
5. Cyst wall thinner.	5. Cyst wall thicker.

As regards refractiveness Wenyon notes *E. histolytica* as being more refractive than *E. coli*. This of course would accord with Schaudinn's highly refractile ectosarc of *E. histolytica*. In my opinion the porcelaneous optical character of *E. coli* is of great differentiating value. Again the deeper staining of *E. coli* in Grams iodine solution, together with the sharp outline of the 8 nuclei in such a preparation, is of value.

AMŒBÆ OF PYORRHŒA ALVEOLARIS

Recently much importance has been attached to certain amœbæ found deep in the pus pockets of affected teeth. They are best obtained after wiping away the superficial pus and then scraping material from the depths of the pockets with a wooden toothpick. They may be emulsified in salt solution but saliva is better for the obtaining of motility. The name is *Entamœba gingivalis* and it is probably the one described under the names *E. buecalis* and *E. dentalis*. It rather resembles *E. histolytica* in having a greenish refractile ectosarc and an indistinct nucleus. Encysted forms have not been observed.

It is open to question whether these amœbæ are the cause or whether various streptococci bring about the condition. A symbiotic relationship may be operative. Emetine seems often of value especially when combined with an autogenous vaccine. The recent enthusiasm for emetine in treatment of pyorrhœa seems to be disappearing.

Entamœba gingivalis varies from 10 to 25μ in diameter. The nucleus is much smaller than those of the intestinal amœbæ and has a distinct nuclear membrane enclosing a deeply stained karysome. On the whole it is poor in chromatin, in this respect rather resembling the histolytica nucleus.

Pseudopod projection is less active than that of *E. histolytica* but more so than *E. coli*. There is no distinction between endoplasm and ectoplasm and the nucleus is indistinct. Encysted forms are very rarely seen and these do not show evidence of reproduction.

FLAGELLATA (MASTIGOPHORA)

In this class of protozoa the adults have flagella for the purposes of locomotion and the obtaining of food.

Some flagellates more or less resemble rhizopods in being amœboid and in having an eetoplasm and an endoplasm. The body is frequently covered by a cuticle (periplast). Some flagellates have a definite mouth part, the cytostome, which leads to a blind œsophagus; others absorb food directly through the body wall. In addition to flagella, some flagellates possess an undulating membrane. All flagellates possess a nucleus and some have contractile vacuoles. The flagellum may arise directly from the nucleus or from a small kinetic nucleus, the blepharoplast (micronucleus or basal granule).

The most important flagellates of man are the hæmoflagellates. Among these we may include the blood spirochætes and the organism of syphilis, which have many resemblances to the spiral forms of bacteria, together with the three genera in which protozoal characteristics are marked, namely, *Leishmania*, *Trypanosoma* and *Trypanoplasma*. In addition we have flagellates in the intestinal canal and in the vaginal secretion. Some authors place the genus *Piroplasma* with the flagellates and there has been controversy concerning the nature of certain projections from these bodies. It would seem preferable, however, to consider them under the Sporozoa.

Spirochæta

The generic term *Spirochæta* is applied to flagellates having a spiral shape, an undulating membrane, and no flagella. This genus is one about which there are two views: one, that the members belong to the bacteria; the other, that they are protozoa. The absence of demonstrable nucleus and blepharoplast makes them apparently vegetable in nature while the variations in thickness, the fact of transmission by an arthropod, and indications of a longitudinal, rather than a transverse division, would indicate protozoal affinities.

It would seem from recent investigations that both methods occur—longitudinal division occurring when there are few organisms in the blood and transverse at the height of the infection.

Minchin has adopted the name *Spiroschaudinnia*, proposed by Sambon, for the parasitic blood spirochætes.

Spirochætes of Relapsing Fevers.—Relapsing fevers are caused by organisms generally considered as protozoal in their nature and belonging to the flagellates.

The generic name *Spiroschaudinnia* is preferred by some to the more commonly accepted *Spirochæta*. East and West African relapsing fever, or tick fever, is caused by *S. duttoni* and the transmission is through the bite of an argasine tick, *Ornithodorus moubata*. Not only does the tick itself become infected by the taking in of blood-containing spirochætes but likewise transmits the infection to its progeny. Leishman considers that when the spirochætes are taken into the alimentary tract of the tick there is a breaking up of the spirochætes into small granules which reach the Malpighian tubules. They also invade the ovary and the ova. It was thought that these granules were the infecting agents and that they were excreted in the fluid of the coxal glands or passed out with the fæces. More recently it has been claimed that these granules have no relation to the infection, which is due to spirochætes as such.

It may be stated that spirochætes as such may be found in the secretion of the coxal glands as well as in the fæces. This coxal fluid

dilutes the thick fæces and makes an emulsion which is smeared out by the body of the tick in the area of the bite puncture.

At any rate this infection of man seems to be the contamination method, the material from fæces and coxal glands being rubbed into the wound made by the tick bite. The ticks hide in the cracks about the old native huts and bite the sleeping inmates. There may be quite a local reaction at the site of the bite.

FIG. 66.—Spirochætæ of relapsing fever from blood of a man. (*Kolle and Wassermann.*)

Spirochæta duttoni has been cultured by Noguchi, by utilizing his methods for culturing the organism of syphilis. In such cultures he has noted longitudinal division rather than transverse, this fact rather favoring a protozoal as against a bacterial nature. This spirochæte is from 24–30 microns long, about 0.45 microns broad and has a corkscrew motility. It is readily transmissible to a number of laboratory animals, as monkeys, white rats, etc. The spirochæte of Northern

FIG. 67.—*Spirochæta novyi.* (*Todd.*)

African relapsing fever, *S. berbera* causes the disease as seen in North Africa and Egypt. It is transmitted by lice, Nicolle and others having shown that the spirochætes make their way from the alimentary tract to the body cavity of the louse. They have shown that the bite alone of an infected louse is innocuous and also that the fæces are noninfective, when injected into monkeys. Emulsions of infected lice, however, when rubbed into wounds, produce the disease in monkeys.

The spirochætes taken in by a louse disappear in a few hours and the insect remains harmless until about the fifth day, when it becomes infectious, and so remains until the twelfth to fifteenth day. Spirochætes reappear in the cœlomic fluid of the louse about the sixth day and continue present until about the twentieth day.

A striking fact is that infection can be brought about a day before spirochætes appear and that after a period of a few days these spirochæte containing lice lose their power to infect. It would seem that the infecting stage was an invisible one. Have we then a symbiosis between a spirochæte and an invisible virus, possibly filterable? Wolbach has shown that certain spirochætes will pass through a Berkefeld filter as spirochætes but this would not affect the possibility of the existence of some granule or chlamydozoal stage. It may be that the infecting stage is not an invisible one but a granule one.

It is by crushing the louse, by scratching or otherwise, that the spirochætes contained in the cœlomic fluid reach and penetrate the wound of the bite. This is therefore a contaminative method of infection.

Mackie has shown that the Indian relapsing fever, which is caused by *S. carteri*, is probably transmitted by the louse, and it is probable that the conditions under which the infection takes place are similar to those occurring with *S. berbera* infections. With the European relapsing fever, bedbugs have been suggested as transmitting agents. The probabilities however are that this infection is transmitted by lice alone.

A relapsing fever of Persia is transmitted by a tick of the genus *Ornithodorus*.

Diagnosis.—In blood examinations we may use the dark field illumination, although the spirochætes stain readily with Wright's stain. The India-ink method is a good one. Hagler recommends smearing out a mixture of one loopful of blood and a collargol preparation made by diluting one part collargol with two parts water, allowing it to stand twenty-four hours, then filtering.

There is great variation in the description of the different spirochætes, and frequently measurements are given for short forms and long forms. They also vary from wave-like lines to corkscrew spirals. Again, different species have different types and different activities of movement. As a rule they are about 20×0.4 microns. Noguchi has recently cultivated the various species of pathogenic human spirochætes by employing a method similar to that used in cultivating the organism of syphilis. He noted longitudinal division in his cultures.

Spirochæta icterohæmorrhagiæ.—This is morphologically like the spirochæte of syphilis. It is present in the blood and urine of cases of Weil's disease but its demonstration is very difficult. For diagnosis we inject the blood of a suspect into a guinea-pig (intraperitoneally) and numerous spirochætes will be present in the liver of the animal. In the U. S. some studies have shown that the infection is quite common in wild rats and, as transmission is by way of the urine, it is probable that the incidence of the disease in trench warfare is connected with the infected rats infesting the trenches. We acquire the infection by ingesting infectious material.

S. vincenti.—This is a very delicate spiral-shaped organism which has been found in conjunction with a fusiform bacillus in a throat inflammation, usually termed Vincent's angina.

S. refringens.—This *Spirochæta* is frequently associated with the *Treponema pallidum* and is common in genital ulcerations. It is thicker, has less regular and more flattened curves and stains more readily. By "dark-ground illumination" it is thicker, of a yellow tint instead of pure white, and moves in its entire length.

Treponema

The genus *Treponema* has no undulating membrane and has a flagellum at each end.

Treponema pallidum (Spirochæta pallida).—This is the cause of syphilis. It was discovered by Schaudinn and Hoffmann in 1905 in various syphilitic lesions and was cultured by Noguchi in 1912. It is characterized by the very geometric regularity of the spirals, which are deeply cut, and in focusing up and down continue in focus (like a corkscrew). They require about thirty minutes to stain distinctly with Giemsa's stain and the attenuated ends or flagella should always be noted before reporting their presence.

Treponemata are found in the cellular areas surrounding the thickened blood-vessels and in the coats of the larger arteries. To stain them in section Levaditi's method is the best.

The India-ink method of Burri is highly recommended. Take one loopful of secretion from a chancre and deposit it on one end of a slide. Emulsify in this a small quantity of Günther and Wagner's ink such as can be obtained by touching the end of a new spatulate toothpick in the ink. Use the toothpick for mixing. I use the method of sticking a fine glass pipette into the underlying corium of the chancre and get serum in that way or by squeezing the chancre afterward. Mix and make a smear as for blood. When dry examine with the oil immersion objective and the treponemata will be found to stand out as white spirals against a dark background. Treponemata often appear as if bent in the middle.

Harrison prefers collargol to India ink. One part of collargol is put in a bottle with 19 parts of water and well shaken. This shaking is repeated. One loopful of the suspected serum and one loopful of the collargol suspension are mixed and smeared out and examined as for the India-ink method.

Cultivation.—*T. pallidum* has been cultivated anaerobically in horse serum by Schereschewsky. The cultures contained other organisms. Muhlens, by growing anaerobically on horse serum agar (1 to 3), claims to have obtained pure cultures. Animal inoculations with this material were negative, however.

Noguchi has cultivated *T. pallidum* under strict anaerobic conditions in a medium of ascitic fluid containing a piece of fresh sterile tissue, preferably placenta. The growth is faintly hazy and does not have an offensive odor. *Spirochæta micro-*

dentium shows similar morphology but the cultures have a foul odor. *Sp. macrodentium* is similar culturally but differs morphologically.

Luetin.—When cultures of *T. pallidum*, grown for one or more weeks in ascitic fluid agar and ascitic fluid, are ground in a mortar, heated to 60°C. for one hour then, with the final addition of 1% trikresol, we have an emulsion called "luetin." This extract produces an allergic reaction on the skin of certain syphilitics (luetin reaction). To carry out the test luetin is introduced intradermally at the insertion of the left deltoid and a control emulsion of agar media injected in the right arm. A negative result shows as an erythema without pain or papule formation. Positive reactions show as papules vesicles or even pustules giving rise to discomfort for several days. Nor only do we have papular and pustular type reactions but also torpid ones (taking ten days or more to develop). While the control side usually becomes normal in forty-eight hours yet in latent and tertiary syphilis the control may show almost as marked a reaction. The term "Umstimmung" is applied to this susceptibility to trauma of the skin of those having tertiary syphilis. Some cases of parasyphilitic infections which are negative to the Wassermann test give a positive luetin reaction. Tertiary yaws cases frequently give a positive luetin reaction. See comparison of Wassermann and luetin statistics.

Noguchi has recently demonstrated *T. pallidum* in all layers of the cerebral cortex except the outermost one in 12 cases out of 70 cases of general paresis examined.

Diagnosis.—In diagnosis either use the dark-ground illuminator or make a thin smear from the sanious oozing after vigorous friction of the chancre with gauze, taking up this blood-stained serum on the end of a slide and smearing the surface of a second slide with the adhering material. It is in most cases more satisfactory to curet the lesion, in this way obtaining material from the areas of the thickened arteries. Fontana's silver staining method is an excellent one.

In the diagnosis of cerebrospinal syphilis we use, in addition to the Wassermann test of the blood, (1) the Nonne-Apelt reaction in which about 1 c.c. of a saturated aqueous solution of ammon. sulphate is added to an equal amount of cerebrospinal fluid. If turbidity or rather opalescence appear immediately, or within three minutes, the test is positive. We now use a ring test. (2) The counting of the lymphocytes in the cerebrospinal fluid. A lymphocytosis occurs in cerebrospinal syphilis, tabes and general paresis. (3) The Wassermann test, using the cerebrospinal fluid instead of blood-serum. (4) The colloidal gold test of Lange. These various examinations of spinal fluid are taken up in detail in chapter on cytodiagnosis and spinal fluid examinations.

T. pertenue.—An organism of similar morphology was first reported by Castellani as present in yaws. It is found in smears and sections as with *T. pallidum*.

A point of distinction between these spirochætes is that the *T. pallidum* is found in abundance in sections from a chancre about the thickened arteries in the croium, while in sections from a yaws nodule the *T. pertenue* is found chiefly in the region of the interpapillary pegs of the Malpighian layer of the epidermis where they bound the papillary layer of the corium.

T. pertenue has been cultivated in the same way as *T. pallidum* and Nichols has infected rabbits by intratesticular injection. A disease of Guam known as gangosa is possibly connected with a tertiary form of yaws. In persons who have had yaws a positive Wassermann reaction seems to be given in a higher percentage than is true for syphilis. Salvarsan is also more specific for yaws than for syphilis.

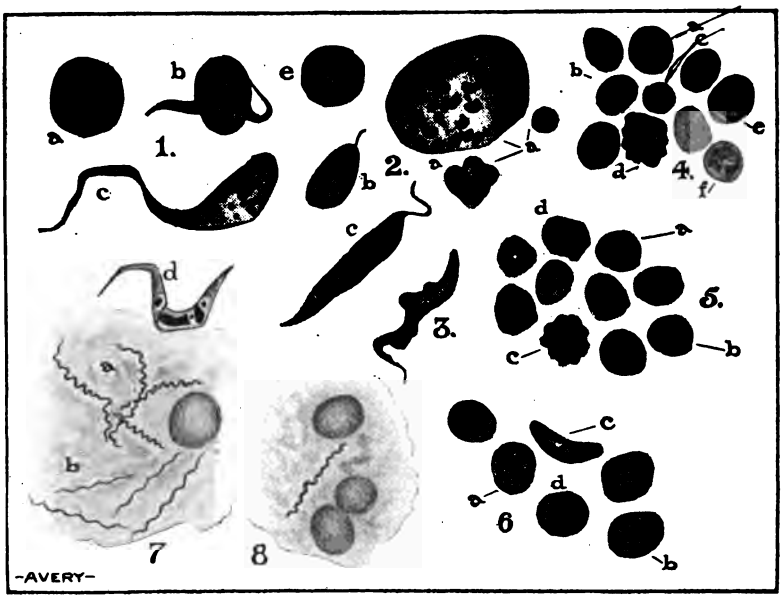


FIG. 68.—Binucleata (Hæmoflagellata and Hæmosporozoa). 1. *Schizotrypanum cruzi;* (*a*) Merozoite just entering r.b.c.; (*b*) fully developed trypanosome form in blood; (*c*) form found in intestine *Conorhinus;* (*d*) form in salivary gland of *Conorhinus;* (*e*) merocyte from the schizogenous cycle in lungs. 2. *Leishmania donovani;* parasites from spleen smear, free and packed in phagocytic cell; (*b*) and (*c*) flagellate forms from cultures. 3. *Trypanosoma gambiense.* 4. *Plasmodium vivax;* (*a*) young schizont; (*b*) uninfected red cell; (*c*) red cell, punctate basophilia; (*d*) merocyte; (*e*) macrogamete; (*f*) adult schizont. 5. *Plasmodium malariæ;* (*a*) half-grown schizont showing equatorial band; (*b*) macrogamete; (*c*) merocyte; (*d*) young schizonts. 6. *Plasmodium falciparum;* (*a*) red cell showing multiple infection; (*b*) young ring from; (*c*) crescent; (*d*) young schizont on periphery of r.b.c. 7. (*a*) *Treponema pallidum;* (*b*) *Spirochæta refringens.* 8. *Treponema pertenue.*

TRYPANOSOMES OF SLEEPING SICKNESS

The African trypanosomiases follow infection with two species of trypanosomes; the more virulent type of the disease, occurring in South Central Africa, being due to *Trypanosoma rhodesiense,* transmitted by *Glossina morsitans* and that of less severe type, but of more general distribution, being due to *T. gambiense* and transmitted

by *Glossina palpalis*. The very important *Trypanosoma brucei*, which is the devastating agent in the African horse, dog and cattle disease, nagana, is also transmitted by *Glossina morsitans* and there exists the opinion that this trypanosome is identical with *T. rhodesiense*. A third human trypanosome is *T. nigeriense*, said to be transmitted by *G. tachnoides*.

These trypanosomes are blood flagellates and are typical of the Binucleata in possessing two chromatin staining areas, the larger and more centrally situated mass being the tropho or macronucleus and the smaller, but more deeply staining one, the kineto or micronucleus (Blepharoplast). Trypanosomes have a fusiform or

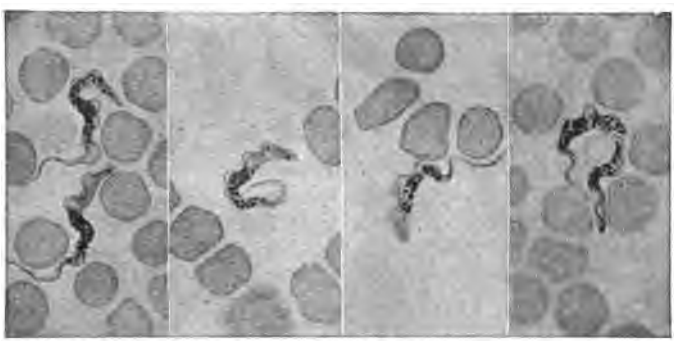


FIG. 69.—*Trypanosoma gambiense* (slide presented by Professor F. G. Novy). (*From Todd.*)

fish-shaped body which stains blue. Near the less pointed, nonflagellated end, usually called the posterior end, is the deeply stained blepharoplast. Behind this is a vacuole and, taking origin from this part of the trypanosome, is the flagellum. This borders an undulating membrane attached to the body and then, carried along to the other extremity, projects free as a long, whip-like flagellum.

In fresh preparations the body of the trypanosome progresses in the direction of its flagellated end, although occasionally it will be observed to move in the opposite direction.

T. gambiense varies much in length and breadth. The normal type, as found in the blood, varies from 14 to 20 microns, while longer forms, 20 to 24 microns, are growth ones and, in the longest ones (23 to 33 microns), we have those preparing to divide longitudinally. The normal short forms are the ones from which the development takes place in the tsetse fly. In width these flagellates are from 1.5 to 2 microns. The blepharoplast is oval and the nucleus situated about the center.

With *T. rhodesiense* the nucleus is typically located almost adjacent to the blepharoplast. As a matter of fact it may require the passage of

this trypanosome through rats to bring out these "posterior nuclear forms," the nuclear location being at times almost entirely that of *T. gambiense*. In addition to the characteristics of nucleus being near the blepharoplast, this trypanosome is more virulent for laboratory animals than *T. gambiense*, agreeing in this respect with the more severe clinical course in man.

When the tsetse fly, *Glossina palpalis*, feeds on a man in whose peripheral circulation there are normal type trypanosomes we have an accumulation of such forms in the middle and posterior portions of the gut. From the eighth to the eighteenth day long, slender forms develop and pass forward into the proventriculus. None of the intestinal forms can cause infection when injected into animals. These proventricular types work their way into the salivary ducts and thence into the salivary glands, where further development takes place. Here we have shorter forms developing, which are similar in morphology to the normal blood type. It is at this stage that the fly becomes infective by the passing of these trypanosomes down the salivary ducts and through the channel in the hypopharynx to the subcutaneous tissues of the person bitten. High temperatures, 75 to 85°F., are favorable to development, while low temperatures, 60 to 70°F., are inimical to development, but do not kill the ingested trypanosomes. This explains the long period which at times elapses before a fly becomes infective. Under favorable conditions a fly becomes infective in twenty to twenty-four days and remains infective the rest of its life, up to 185 days. The infection is not transmitted to the pupa. This is an inoculative, cyclical or indirect type of infection. It is usually considered that a tsetse fly whose proboscis has just been contaminated with trypanosome blood is capable of transferring the infection for a few hours. This would be a mechanical or direct method of infection and such power for infection only lasts for a few hours.

When tsetse flies feed on animals infected with trypanosomes only from 2 to 6% become infective. Again, it has been shown that where the wild animals on which tsetse flies feed may show an infection of from 16 to 50% yet not more than two out of every 1000 tsetse flies, caught and tried out on susceptible animals, show themselves infective.

Both of the human trypanosomes of Africa have been cultured by using the N.N.N. medium in which rat's blood was substituted for that of the rabbit. Human blood will also serve as a substitute. Growth however is not constant.

For the laboratory diagnosis we may use peripheral blood with some thick film method. The examination of preparations from the peripheral blood is usually very discouraging. Very much better results (in fact some prefer this method to any other) can be obtained by taking 10 to 20 c.c. of blood into about 25 c.c. of citrated salt solution, centrifuging two or three times and examining the sediment of the

third centrifugalization. Dutton and Todd prefer to centrifuge citrated blood and to collect the leukocyte layer for examination as is done in opsonic work.

The English workers usually prefer the gland puncture method, using a sterile but dry hypodermic needle. Water in the needle distorts both Leishman bodies and trypanosomes.

In the sleeping sickness stage trypanosomes can almost constantly be found in the cerebrospinal fluid.

Some prefer to inoculate susceptible animals, particularly the guinea-pig or monkey, with blood or gland jüice from the suspected case. A very satisfactory material is an emulsion from an excised gland which may be inoculated intraperitoneally into white rats. The further course, after animal inoculation, is the examination of the blood of these animals for trypanosomes. Usually at the time the guinea-pigs die we find numerous trypanosomes.

Other tests are (1) Trypanolysis, when unheated suspected serum and trypanosomes are incubated together for one hour. Normal serum may occasionally cause disintegration and treated cases give it in only about 45% of cases. Unfavorable untreated cases give it in about 80% of cases.

(2) The so-called auto-agglutination test is not of much value. In this the red cells of the blood of a trypanosomiasis case come together in clumps when one makes a wet preparation. It is not a rouleaux formation. (3) The attachment test is made by making a mixture of inactivated serum, leukocytes and trypanosomes and allowing them to be in contact for twenty minutes. A positive test shows attachment of the trypanosomes to the leukocytes.

Of the more important trypanosome diseases of animals may be mentioned:

Trypanosoma brucei.—This trypanosome causes a surely fatal disease in horses and one from which few cattle recover. It is called "nagana" or the fly disease, from being transmitted by the tsetse fly, *Glossina morsitans*. All animals except man and possibly the goat seem susceptible. The disease is characterized by fever, œdematous areas about neck, abdomen and extremities, progressive anæmia and emaciation. It is an important disease of domesticated animals of many parts of Africa.

Trypanosoma evansi.—This is the cause of a very fatal disease of horses in India and the Orient and known as "surra." It also affects camels and even cattle. It is thought to be transmitted by biting flies (*Scmoxys*). The symptoms are fever, emaciation, œdematous areas and great muscular weakness.

Trypanosoma equinum.—This trypanosome causes a fatal disease in horses in South America. There is paralysis of the hind quarters of the horse which gives the disease the name "mal de caderas."

Trypanosoma equiperdum.—This trypanosome causes a disease of horses in many parts of the world. It is known as "dourine" and is transmitted by coitus. The genital organs show marked œdema which is followed by anæmia and paralysis.

Trypanosoma dimorphon.—This trypanosome causes a disease of horses in Gambia. It is also found in horses and cattle in other parts of Africa. The parasite shows marked variation in morphology.

Trypanosoma lewisi.—Rats in many parts of the world show this infection which is rarely fatal to them. It is transmitted by the rat flea by a process of regurgitation. It can also be transmitted by the rat louse.

There are many trypanosomes in birds, fish, frogs, etc.

Schizotrypanum cruzi (Trypanosoma cruzi).—In 1909, Chagas reported the finding of a flagellate in the intestines of *Conorhinus megistus* or, more properly, *Lamus megistus*. He was also able to transmit the flagellate to laboratory animals and could culture it on blood agar.

In investigating the matter of the importance of this flagellate, *Schizotrypanum cruzi*, in Minas Geraes, Brazil, where the bug was present in great numbers in the cracks of the houses of the poor, he associated this flagellate infection, which he at first considered trypanosomal, with a disease of the children of that section.

The bug is a vicious feeder and, from its biting chiefly about the face, has been called barbiero or barber by the natives. Both the male and female of *Lamus* bite and can transmit the disease and although the parasite is not transmitted hereditarily the nymph is capable of sucking blood and becoming infected.

It requires several months for the insect to go through the egg, larval and pupal stage to maturity. Some consider this bug to belong to the genus *Triatoma*. The insects may live for more than a year and tend to remain in the same house where they may have become infected but leave such house if it be abandoned by man. Brumpt thinks that the bedbug may also transmit the disease.

FIG. 70.—*Schizotrypanum cruzi* in blood of child with acute type of Brazilian trypanosomiasis. (*MacNeal from Doflein after Chagas.*)

S. Cruzi is found in the blood of children during the acute febrile stage but at other times in children, and as a rule in adults, it is rarely present in the peripheral blood. The early blood forms are narrow and very motile. They increase in size and slacken in motility when they become about 20 microns long. *S. cruzi* is characterized by a very large blepharoplast. Dividing forms are never seen in the blood. The common site of multiplication is in the cells of the voluntary muscles and heart and also in the cells of the central nervous system, adrenals, thyroid and bone marrow. In these tissues the flagellate takes on a rounded form and undergoes binary division. Continued

division converts the infected cell into a cyst. It is this process going on in various important structures that accounts for the extreme variation in symptomatology and pathology.

Chagas thinks that the gametes for the cycle in *Lamus* arise from parasites developing in the lungs of the vertebrate host. Flagellated parasites enter the lungs, lose the flagellum and become oval in shape, later on dividing into 8 parts. These assume an elongated form and enter the red cells of the host. The forms taken up by *Lamus* multiply in the intestine and then pass to the salivary glands after about eight days. The bug is then infectious when it bites. Brumpt notes that infection may occur from inoculation of the fæces passed by the bug, especially through the conjunctiva.

Trypanoplasma

The genus *Trypanoplasma* has a rather large blepharoplast, from which arise two flagella. One extends forward as a free anterior flagellum, while the other projects posteriorly, running along the border of the undulating membrane. This genus is not known for man.

Leishmania

The parasites which cause a general infection in kala-azar and leishmania infantile splenic anæmia but a local one in oriental sore are usually separated as distinct species,[1] *Leishmania donovani* for kala-azar,[2] *L. infantum* for infantile splenic anæmia and[3] *L. tropica* for oriental sore.

These parasites are grouped with the hæmoflagellates and occur in their vertebrate hosts exclusively as small, oval, cockle-shell-shaped bodies, measuring 2.5 × 3.0 microns. The protoplasm stains a faint blue and contains a rather large trophonucleus which is peripherally placed and gives the appearance of the hinge of the cockle shell. Besides this macronucleus we have a second chromatin staining body which is often rod-shaped and set at a tangent to the larger nuclear structure. It is called the blepharoplast or micronucleus and stains a more intense reddish than the rather fainter stained pinkish macronucleus. One or more vacuoles are common in the cytoplasm.

Some consider these nonflagellated bodies, which are usually found packed in endothelial cells of spleen, liver, lymphatic glands and bone marrow, as resting stages, the flagellate existence occurring in some other host than its vertebrate one. Patton has carried on an immense amount of experimental work with the bedbug and has noted the development of flagellate forms from the fifth to the eighth days in bugs which fed on kala-azar patients showing Leishman bodies in their peripheral circulation. If the bugs are allowed a second feeding after the infecting blood meal the flagellates disappear within twelve hours, so that for full development in the bedbug a single feeding is requisite. He states that the flagellate forms change to post-flagel-

late ones by the twelfth day. At the same time, although much evidence exists in favor of the bedbug as host for the flagellate forms, it has not been shown experimentally that the bedbug is definitely connected with the transmission of the disease.

Donovan is disposed to incriminate *Conorrhinus rubrifasciatus* as the transmitting agent and furthermore he feels that there has not been sufficient investigation of mosquitoes along this line.

In the regions where leishmaniasis of infants occurs there is also found a similar disease of dogs and Basile has claimed that the disease is transmitted from dog to dog by the dog flea.

FIG. 71.—*Leishmania tropica.* Smear from granulation tissue of Delhi boil or oriental sore. (*MacNeal from Doflein after J. H. Wright.*)

As the dog has been regarded by some as the reservoir of the virus so naturally the transmission of the disease from dog to child through the flea has been considered. Wenyon, however, tried to infect two young dogs with great numbers of fleas which had previously fed on dogs infected with canine leishmaniasis and at autopsy, five or six weeks later, was unable to find parasites in smears from spleen, liver or bone marrow and did not succeed in obtaining cultures from this material inoculated into tubes of N. N. N. medium.

As regards oriental sore Wenyon has found that bedbugs and *Stegomyia* will feed from the sores and take up parasites which develop into flagellate forms in the gut of the insects.

Proof of transmission by these agents, however, is lacking and others are inclined to suspect the house fly or some species of moth midge.

In Brazil there exists some evidence that the cutaneous leishmaniasis found there may be transmitted by species of the tabanid family.

It must be understood that there is always a suspicion that the flagellate forms noted in arthropod experiments may be those of non-pathogenic herpetomonad or crithidial species as such forms are common in arthropods and are difficult to distinguish from the flagellate stage of Leishman bodies.

The genera *Herpetomonas* (*Leptomonas*) and *Crithidia* are frequently found in the alimentary tract of insects and have caused confusion in the search for developmental forms of various pathogenic flagellates in transmitting insects. In *Herpetomonas*, of which the type species is *H. muscæ domesticæ*, the body is spindle-shaped with a rather blunt flagellar end and an attenuated anterior end. In *Crithidia* both extremities are pointed and the blepharoplast is situated toward the center quite near the trophonucleus. In *Herpetomonas* the blepharoplast is near the rather blunt flagellar extremity at some distance from the nucleus.

There is no undulating membrane in *Herpetomonas* and only a slightly developed one in *Crithidia* while *Trypanosoma* has a fully developed one. In *Herpetomonas* the blepharoplast is near the flagellated end and at a distance from the trophonucleus. In *Crithidia* the blepharoplast is more posterior and near the macronucleus but still anterior to it while in *Trypanosoma* the blepharoplast has moved so far posteriorly as to pass the nucleus and be located at the posterior extremity. The flagellated *Leishmania* is morphologically herpetomonad.

Very definite is our knowledge of the cultural forms of *Leishmania*. Rogers first cultured material from splenic juice of kala-azar patients in 10% sodium citrate solution at a temperature of 17° to 24°C. The medium was slightly acidulated with citric acid. There was no satisfactory development at blood temperature. In forty-eight hours the oval parasites have developed into herpetomonad flagellates, from 20 to 22 microns long by 3½ microns broad, with a 20-micron flagellum which takes origin from the blunt anterior end of the body near the blepharoplast. The peripheral blepharoplast and centrally placed macronucleus are at a distance from one another as opposed to the approximation of the crithidial blepharoplast to the centrally placed nucleus in a body with pointed anterior end.

Formerly it was thought that there were differences in the three species of *Leishmania* from the standpoint of growth on various culture media, *L. donovani* not growing on N. N. N. medium while *L. infantum* grew well on N. N. N. medium but not in citrated blood. It is now known that both species will grow on these two media.

It is absolutely essential in culturing *L. donovani* or *L. infantum* that the blood agar or citrated blood be sterile, as any bacterial contamination prevents growth. With the parasite *L. tropica*, however, bacterial contamination does not inhibit development and statements have even been made that growth is favored by a staphylococcal symbiosis. *L. tropica*, it would seem, will develop into flagellated forms in cultures at 28°C. while it will be remembered that Rogers in his original experiments failed to obtain other than commencing signs of division at 27°C., 22°C. being the temperature necessary for the development of flagellate forms.

L. tropica from South American cutaneous leishmaniases seems to grow more luxuriantly on N. N. N. medium than does that of oriental sore of Asia and Africa.

While differences in development on different culture media may obtain not only with different species but with different strains of the same species, it would appear that such variations cannot be utilized as a means of separating the three species.

With animal inoculations we formerly thought that the parasite of kala-azar could be differentiated from that of infantile leishmaniasis by the fact that dogs could not be infected with *L. donovani*, while they were susceptible to infections with *L. infantum*. Recently Donovan and Patton have successfully inoculated dogs with kala-azar splenic material. Patton found the parasites in the liver, spleen and lymphatic glands as well as bone marrow of the inoculated dogs. Consequently we cannot separate the two visceral leishmaniases from a standpoint of susceptibility of the dog. Monkeys are susceptible to both diseases.

As regards separating oriental sore from the visceral leishmaniases Gonder has shown that white mice may be infected with both kala-azar and oriental sore, there being produced in each case a general infection with the presence of parasites in spleen and liver. A point of difference, however, is that the oriental sore mice develop lesions on feet, tail and head which was not observed with the kala-azar mice. There are some reasons for thinking that in human cutaneous leishmaniasis a generalized infection may precede the local manifestations.

A very interesting point is that the dogs in India never show a natural infection with *L. donovani* while in the regions where *L. infantum* is responsible for human infections the natural infection of dogs is not uncommon, indeed many think the dog the reservoir of virus for both *L. infantum* and *L. tropica*. It has been suggested that the dogs of India, where kala-azar prevails may be immune.

As regards morphology it is usually stated that the parasites of the three species of *Leishmania* are practically identical. In cultures it has been noted that the flagella of *L. tropica* are longer and more twisted than those of *L. infantum*. Again it has been observed that the parasites of the Oriental and South American skin lesions may at times show a flattened or band-like trophonucleus instead of the constant round or oval one of the visceral leishmaniases.

Escomel has reported the finding of flagellated *Leishmania* in the South American sores.

Laboratory Diagnosis.—The leukæmias can be easily differentiated by the blood picture, an important matter because the spleen of splenomyelogenous leukæmia is very friable and the danger from splenic puncture is far greater in this condition than in kala-azar. Banti's disease with its leukopænia shows a rather similar blood picture and

can only be surely differentiated by the finding of Leishman bodies in kala-azar.

Malta fever, typhoid and the paratyphoids are best differentiated by blood cultures or agglutination tests.

Until recently it was recommended that for diagnosis our best procedure was to make a splenic puncture. Manson and others have pointed out the dangers from splenic puncture in kala-azar and have rather preferred puncture of the liver, although recognizing that the chances of obtaining parasites from a liver puncture, are less than from a splenic one.

Statistics have been given where a mortality approximating 1% has followed spleen puncture. Bousfield, however, using an all glass syringe with a 1½-inch needle did not have a fatality in 120 spleen punctures.

For diagnosis the spleen or liver juice, rather than pure blood, is smeared on a slide and stained by some Romanowsky method, preferably that of Giemsa.

Cultures on N. N. N. medium can also be made.

One should always first examine a smear of the peripheral blood for parasites in polymorphonuclear or large mononuclear leukocytes. The Sudan Commission found Leishman bodies in the peripheral blood of 13 out of 15 cases so examined, but rarely did they find more than one parasite-containing leukocyte to a slide.

Quite recently Wenyon and others have noted the desirability of culturing the peripheral blood in N. N. N. medium. Diagnosis may be made in this way, provided one waits from two to three weeks before reporting negatively as to the presence of flagellated *Leishmania* in the cultures. As before stated, strict asepis and a room temperature are requisite for flagellate development.

It has been noted that artificial pustulation might assist in diagnosis by giving a multitude of polymorphonuclear leukocytes for examination for phagocytized *Leishmania*.

Cochran has recently noted the advisability of excising a lymphatic gland and making gland smears to examine for *Leishmania*. Others have reported success with gland puncture as utilized in the glands of trypanosomiasis.

NOTE.—Darling has reported from Panama a protozoon somewhat like *Leishmania* in which the cells of lungs, liver, spleen, and lymphatic glands contained numerous parasites about 3 to 4μ in diameter, slightly oval in outline, and containing a large and small chromatin staining mass. He has given it the name *Histoplasma capsulata*.

Intestinal Flagellates

These parasites of the intestinal tract are separated according to the number of their flagella.

These flagella can easily be counted in a preparation mounted in Gram's iodine solution. For this purpose I take a clean slide and make a vaseline line across it about 1 inch from the end. A drop of the iodine solution is placed on the slide about ½ inch from the vaselined line and a suitable portion of the fæces to be examined is emulsified in it. The edge of a square cover-glass is then applied to the vaselined line and allowed to drop on the preparation. By pressure suitable thicknesses of fluid can be examined. There is an absence of current motion. Better, when accessible, is it to use the dark-field illuminator as in this way the flagella are distinctly brought out. The India-ink method is also applicable. Staining of smears by Giemsa's method, following fixation in methyl alcohol or 5% formalin solution is more satisfactory for flagellates than for amœbæ, which, as before stated, should be fixed in moist smears and stained by hæmatoxylin. This method of mounting in iodine solution, however, is the one I always use for encysted amœbæ.

The intestinal flagellates are classified according to number of flagella, absence or presence of an undulating membrane and of a blepharoplast. Three of these flagellates are but rarely found in the stools and seem to be of little importance. They are (1) *Cercomonas*, which has a single nucleus with one free flagellum and a second one which turns backward to be attached to the body and then projects posteriorly as a second free flagellum, (2) *Bodo*, which has a single nucleus, but two anteriorly projecting flagella and (3) *Prowazekia* which has, besides the nucleus, a blepharoplast from which arise two flagella. These flagellates can be cultivated on media used for the cultural amœbæ and it is thought by some that they at times show amœbæ-like stages.

There is an organism, supposed to belong to the moulds, which may be mistaken for an encysted flagellate. It is called **Blastocystis hominis** and has a large central vacuole with a refractile narrow rim which contains one or more nuclei. When stained by Giemsa's stain the central part is very faintly stained while the rim is deep blue.

Trichomonas intestinalis.—This is a very common parasite in diarrhœal stools but as to its pathogenicity there is much doubt. It is pear-shaped and about 9 by 14 microns. There are three flagella projecting anteriorly with a fourth one bordering an undulating membrane and projecting posteriorly. It has a cytostome near the nucleus. There is also a *T. vaginalis* which is found in vaginal secretion of acid reaction, disappearing when the reaction becomes alkaline as at the time of menstruation. It is somewhat larger than the intestinal form and is not infrequently found in urine.

Tetramitus mesnili.—This flagellate differs from the preceding one in not having an undulating membrane or fourth flagellum. The three anteriorly projecting flagella are long and slender. There is a very prominent long slit-like cytostome within which is a flagellum. The nonflagellate end is very much attenuated.

This parasite has been reported not infrequently as a cause of diarrhœal conditions since its first reporting by Wenyon in 1910.

All the above-mentioned flagellates are found in the large intestines especially in the region of the cæcum.

Lamblia intestinalis.—These parasites are about $10 \times 15\mu$ and have a pear-shaped body with a depression at the blunt anterior end. This depression enables the flagellate to attach itself to the summit of an epithelial cell. Around the depression are three pairs of flagella which are constantly in motion. Another pair of flagella project from either side of the blunt little tail-like projection. When stained, the parasites have a pyriform shape with two chromatin staining areas on either side of the anterior end.

In motion they have a slow tumbling sort of progression. These parasites live in the upper portion of the small intestines. The cysts are oval and show the folded flagellate within. There is an appearance of two curved lines and two dots. This infection is frequently associated with a debilitating diarrhœa. Some cases show marked nervous symptoms. In examining the stools of 384 cases, who had practically all been in Gallipoli or Egypt, Woodcock and Penfold, in the King George Hospital, found 98 infected with protozoa as follows: *Lamblia*, 22; *Trichomonas*, 14; *Tetramitus* (*Macrostoma*), 11; *E. coli*, 57; *E. histolytica*, 8; *Isospora*, 10.

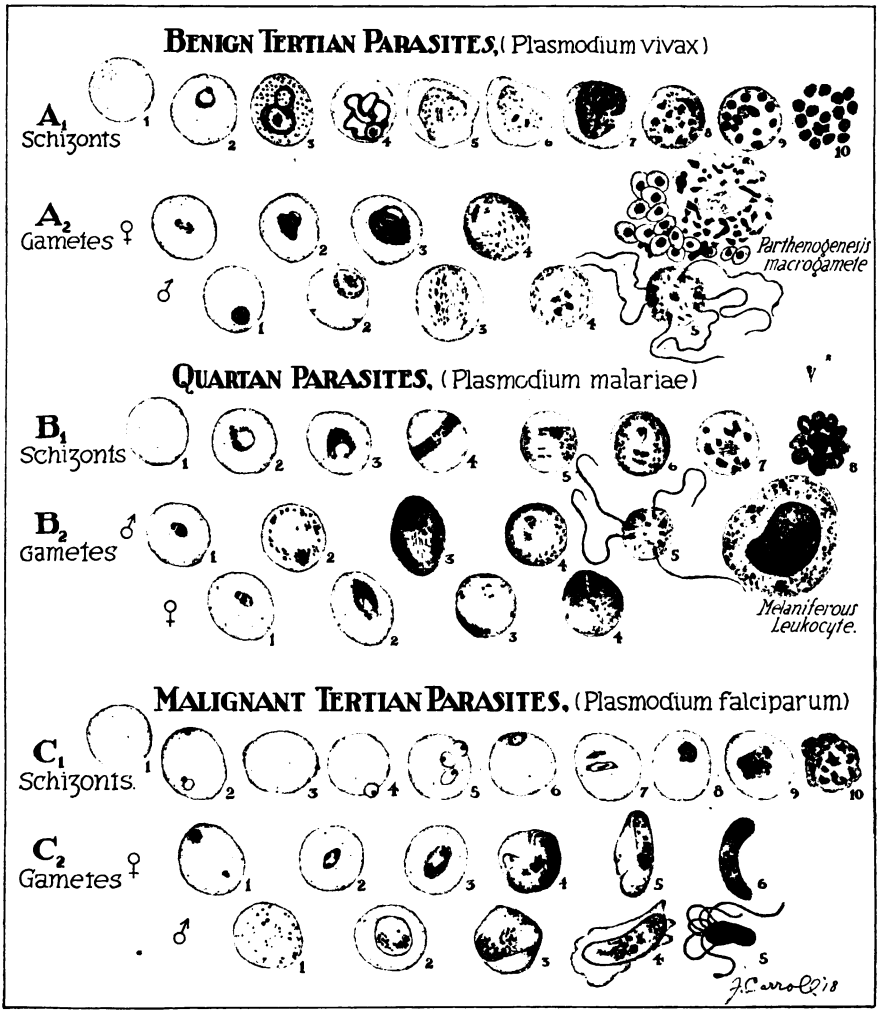
Infusoria (Ciliata)

The Infusoria are the most highly developed of the Protozoa.

The bodies of Infusoria are oval and may be free or attached to a stalk-like contractile pedicle, as with *Vorticella*, or they may be sessile. The cilia, which are characteristic, may be markedly developed around the cytostome (mouth) and serve the purpose of directing food into the interior, while others act as locomotor organs. The body is enveloped by a cuticle which may only have one opening or slit, to serve as mouth; or it may have a second one, a cytopyge or anus. Usually the fæcal matter is ejected through a pore which may be visible only when in use. They usually have a large nucleus and a small one. Infusoria tend to encyst when conditions are unfavorable (as when water dries up in a pond). When the cilia are evenly distributed over the entire body of the ciliates we have the order Holotricha; when ciliated all over, but with more prominent cilia surrounding the peristome, we call the order Heterotricha. It is to this order that the Infusoria of man belong.

Balantidium coli.—This is the only ciliate of importance in man. It is a common parasite of hogs. It is from 60 to 100μ long by 50 to 70μ broad, and has a peristome at its anterior end which becomes narrow as it passes backward. It has an anus. The ectosarc and the endosarc are distinctly marked. The cuticle is longitudinally striated.

PLATE OF MALARIAL PARASITES



(*To face page* 309)

DESCRIPTION OF PLATE OF MALARIAL PARASITES

Benign Tertian Parasites

A1. *Schizonts.* 1. Normal red cell. 2. Young ring form. 3. Amœboid or figure-of-eight form showing Schuffner's dots. 4. Amœboid form showing increased chromatin (twenty-four to thirty hours). 5. Segmentation of nucleus. 6. Nuclear halves further apart, red cells enlarged and pale. Further division of nucleus. 8. Unusual division form. 9. Typical merocyte. 10. Rupture of merocyte liberating merozoites.

A2. *Female gametes.* 1. Young form showing solid instead of ring-form staining. 2. Half grown form. 3. Rapidly growing form with compact nucleus and clear vacuolated zone. 4. Full grown macrogamete showing excentrically placed chromatin and much pigment in deep blue stained protoplasm. *Male gametes.* 1. Young form similar to female one. 2. Half grown form showing central chromatin. 3. Full grown microgametocyte showing large amount of centrally placed chromatin with light blue protoplasm surrounding. 4. Division of chromatin occurring in microgametocyte and developing in wet preparation. NOTE.—Chromatin division in gametes does not take place until blood is withdrawn. 5. Spermatozoon like microgametes developing from the microgametocyte. This only occurs in wet preparations or in the stomach of the mosquito.

Quartan Parasites

B1. *Schizonts.* 1. Normal red cell. 2. Young ring form. 3. Older ring form. 4. Narrow equatorial band. 5. Typical band form. 6. Oval form showing division of chromatin. 7. Early stage merocyte. 8. Daisy form merocyte.

B2. *Male gametes.* 1. Young solid form. 2, 3, 4. Developmental stages microgametocytes. 5. Flagellated body in wet preparation showing microgametes developing from microgametocytes. *Female gametes.* 1. Young ring form. 2. Somewhat older stage. 3 and 4. Mature macrogametocytes (same as benign tertian).

Malignant Tertian Parasites

C1. *Schizonts.* 1. Normal red cell. 2, 3, 4, 5, 6. Young ring forms. These are hair-like rings and are the only forms besides crescents to be found in the peripheral blood. In very heavy infections or in smears from spleen the following forms are found. 7. Beginning division of chromatin. 8 and 9. Further division. 10. Merocyte.

C2. *Female gametes.* 1 and 2. Young macrogametes. 3. Older stage. 4. Development in red cell. 5 and 6. Fully developed female crescents showing clumping of pigment and rich blue colour. *Male gametes.* 1 and 2. Developing forms. 3 and 4. Fully developed microgametocytes. 5. Flagellated body developed in wet preparation.

These parasites cause an affection similar to dysentery and may bring about a fatal termination. It is almost impossible to escape noticing the actively moving bodies if a fæcal examination is made. When encysted they are round.

Another ciliate, the *B. minimum*, 25×15μ, has also been reported for man.

Nyctotherus faba has a kidney-shaped body and is about 25 by 15μ. It has a large contractile vacuole at the posterior end. It has a large nucleus in the center with a small fusiform micronucleus lying close to it. It has only been reported once for man.

Sporozoa

This class of Protozoa gets its name from the method of reproduction —sporulation. These parasites rarely show binary fission. While the sporozoa are found within cells, in the tissues and in internal cavities, as intestine and bile ducts, yet it is as inhabitants of the blood that they have their greatest importance for man—these are known as Hæmosporidia. A sporozoon may be either naked or amœboid or be covered with a distinct cuticle.

Note.—Sporozoa are divided into two subclasses—the Telosporidia and the Neosporidia. In the former the vegetative activity of the protozoon goes on to full growth at which time the reproductive activity commences. With the Neosporidia, however, the growth and reproduction go on at the same time.

Among the Telosporidia we have the orders Gregarinaria, Coccidiaria, and Hæmosporidia.

Gregarines are chiefly parasites of arthropods and worms and are not known for man or the higher vertebrates.

The subclass Neosporidia is practically of no importance in human parasitology, only the order Sarcosporidia having been reported for man. From an economic standpoint, however, the order Myxosporidia is of great importance—*Nosema bombycis* being the cause of pebrine, a disease destructive to the silkworm.

Coccidiaria

The parasites of the order Coccidiaria are almost exclusively found in the intestines and in the organs connected with it. In the vegetative stage it lives within an epithelial cell, which it destroys. Afterward it falls into the lumen lined by this epithelial cell and sporulates, either by the method of schizogony or sporogony.

Owing to their egg-like shape, coccidia have often been considered as the ova of intestinal parasites, and *vice versa*. Upon swallowing an oöcyst with its contained sporozoites the membrane of the oöcyst is digested in the duodenum and the sporozoites liberated. They enter epithelial cells, as of intestine, and reproduce by

schizogony. After a varying number of nonsexual cycles sporogony commences, sporonts being produced instead of schizonts. The female sporont is fertilized by the microgamete which is an elongated body provided with two flagella. These microgametes are formed from the male sporont and when thrown off from the periphery they enter (usually a single one) the macrogamete. After fertilization a resistant membrane is formed and the term oöcyst is used. Within the oöcyst are found smaller cysts, the sporocysts, in which the sporozoites are formed.

The cycle is very similar to that of malaria except that no arthropod host is required for the sexual cycle. The spores which are formed in schizogony are known as merozoites.

Merozoites may best be distinguished from sporozoites by the presence of a nuclear karyosome, this being absent in sporozoites. In *Eimeria* we have the oöcyst containing four sporocysts with two sporozoites in each sporocyst while in *Isospora* we have an oöcyst containing two sporocysts with four sporozoites in each.

Eimeria stiedæ.—This sporozoon is usually known as the *Coccidium cuniculi* or *C. oviforme.* It is most frequently found in the epithelium of the bile ducts. It has very rarely been reported for man. In these cases (about five) cysts of the liver have been found containing coccidia. The parasite is about $40\times20\mu$, and is oval in shape with a double outlined integument. The sporozoites, which form inside, are falciform in shape. These escape and enter fresh epithelial cells, and thus the process of schizogony goes on. The parasites of the liver are larger than those found in the intestines, these latter being only about $30\times15\mu$. In the fæces the form most often found is the oöcyst, about $40\times20\mu$. Infection takes place by ingestion of the oöcyst.

Wenyon states that the *Eimeria* oöcysts, found in cases from Gallipoli, are round instead of the usual oval. They are about 20 microns in diameter and contain four sporocysts, 10×7 microns, each of which has two sporozoites with one or two highly refractile residual bodies.

Isospora bigemina.—This parasite, formerly called the *Coccidium bigeminum,* lives in the intestinal villi of dogs and cats. It is about $12\times8\mu$ and shows a highly refractile envelope (oöcyst) containing two biscuit-shaped sporocysts within each of which are four sporozoites. It has been reported for man three times.

Cases from the Gallipoli district also showed oöcysts of *Isospora* with two sporocysts and four sporozoites in each.

Hæmosporidia

Of the Sporozoa found in the blood (Hæmosporidia), the **malarial parasites** are the only ones connected with disease in man.

There are at least three species of animal parasites which produce human malaria, *Plasmodium vivax,* the cause of benign tertian, *P. malariæ* of quartan and *P. falciparum* of æstivo-autumnal. These parasites belong to the hæmamœba type of the order Hæmosporidia, of the class Sporozoa and of the phylum Protozoa.

This type of Hæmosporidia is characterized by invasion of red cells, amœboid movement, pigment production and the extrusion of

flagellum-like processes from the male sporont after the blood is taken from the animal and allowed to cool.

Other Hæmosporidia which are very important in diseases of domesticated animals, but not for man, are those of the **piroplasm type.**

These parasites of the red cells do not produce pigment and do not "exflagellate." It is to parasites of this type that some authorities have ascribed the cause of blackwater fever, a condition undoubtedly connected with malaria.

It has been thought proper by some to consider the malarial parasites as belonging to two genera, the genus *Plasmodium*, characterized by round sexual forms and including *P. vivax* and *P. malariæ* and the genus *Laverania*, characterized by crescent-shaped sexual forms and including but one species *L. malariæ*, that of æstivo-autumnal malaria.

In addition to man, infections with parasites of a similar nature are found in monkeys (*Plasmodium kochi;* the sexual forms alone seem to be present), in birds (*Hæmamœba relicta;* this organism is usually designated *Proteosoma*). An infection of crows and pigeons of like nature is *Halteridium*. Numerous hæmosporidia have been reported for bats, various other mammals, tortoises, lizards, etc.

The life history of the malarial parasite is one of the most interesting chapters in medicine. Laveran discovered the parasite in 1880. In 1885, Golgi noted that sporulation occurred simultaneously at time of malarial paroxysm. Koch, Golgi, and Celli demonstrated existence of different species for different types of fever. King and Laveran (1884) considered possibility of mosquito transmission. Manson (1894) formulated hypothesis that gametes were destined to undergo development in the mosquito from observing that flagellated bodies only appeared some time after the blood was withdrawn.

Ross (1895) demonstrated that flagellation takes place in the stomach of the mosquito. McCallum (1897) saw fertilization of macrogametes by microgametes of *Halteridium*. Opie recognized differences in sexual characteristics.

Ross (1898) demonstrated life cycle of bird malaria (*Proteosoma*), showing formation of zygotes and presence of sporozoites in salivary glands. Grassi and Bignami proved the cycle for Anophelinæ for human malaria. In 1900 (Sambon and Low), infected mosquitoes from Italy were sent to London, where, by biting, they infected two persons.

Life History of the Malarial Parasite

Malaria can be transmitted by subcutaneous or intravenous injection of the blood of a patient with the disease into a well person, the same type being reproduced.

Such a method of transmission is only of scientific interest and the regular method is as follows: An infected anopheline at the time of feeding on the human blood introduces through a minute channel in the hypopharynx the infecting sporozoite of the sexual cycle.

When man is first infected by sporozoites we have starting up a nonsexual cycle (schizogony) which is completed in from forty-eight to seventy-two hours, according to the species of the parasite. The falciform sporozoite bores into a red cell, assumes a round shape and continues to enlarge (schizont). Approaching maturity, it shows division into a varying number of spore-like bodies. At this stage the parasite is termed a merocyte. When the merocyte ruptures, these spore-like bodies or merozoites enter a fresh cell and develop as before.

Malarial Toxin.—At the time that the merocyte ruptures it is supposed that a toxin is given off which causes the malarial paroxysm.

Rosenau, by injecting, intravenously, filtered blood, taken from a patient at the time of sporulation of the parasites caused a malarial paroxysm. No parasites developed later. Another man who received a small amount of unfiltered blood showed a slight paroxysm and four days later showed parasites in his blood. Hence the parasite will not pass through the pores of a Berkefeld filter.

Schizogony.—The nonsexual cycle goes on by geometric progression from the first introduction of the sporozoite, but it is usually about two weeks before a sufficient number of merocytes rupture simultaneously to produce sufficient toxin for symptoms (period of incubation).

This cycle is termed *schizogony*. It is considered that there must be severa hundred parasites per cubic millimeter sporulating to be capable of producingl symptoms.

Gametes.—After a varying time, whether by reason of necessity for renewal of vigor of the parasite by a respite from sporulation, or whether from a standpoint of survival of the species, sexual forms (gametes) develop. Some think that sporozoites of sexual and nonsexual character are injected at the same time. It is usually considered, however, that sexual forms develop from preëxisting nonsexual parasites. The developing gametes are often termed sporonts. Strictly, the sexual parasites in the blood should be called gametocytes. The gametes take about twice as long to reach maturity as schizonts. The life of a crescent has been estimated as about ten days and that of the gametes of benign tertian and quartan about one-half this period.

Sporogony.—The gametes show two types: the one which contains more pigment, has less chromatin, and stains more deeply blue is the female—a macrogametocyte; the other with more chromatin, less pigment, and staining grayish green or light blue is the male—a microgametocyte. When the gametes are taken into the stomach of the Anophelinæ, the male cell throws off spermatozoa-like projections, which have an active lashing movement and break off from the now useless cell carrier and are thereafter termed microgametes. These fertilize the macrogametes and this body now becomes a zygote. (Following nuclear reduction with formation of polar bodies the macrogametocyte becomes a macrogamete.) This process of exflagellation can be observed in a wet preparation under the microscope. There is first seen a very active movement of the pigment of the male gamete and finally

long delicate bulbous-tipped flagellum-like processes are thrown off (exflagellated) and push aside the red cells by their progressive motion. MacCallum saw a female *Halteridium* fertilized by the microgamete, after which it was capable of a worm-like motion (vermiculus or oökinete).

By a boring-like movement the vermiculus stage of the zygote goes through the walls of the mosquito's stomach, stopping just under the delicate outer layer of the stomach or mid-gut. In three or four days after fertilization the zygote becomes encapsulated and is then often called an oöcyst. It continues to enlarge until about

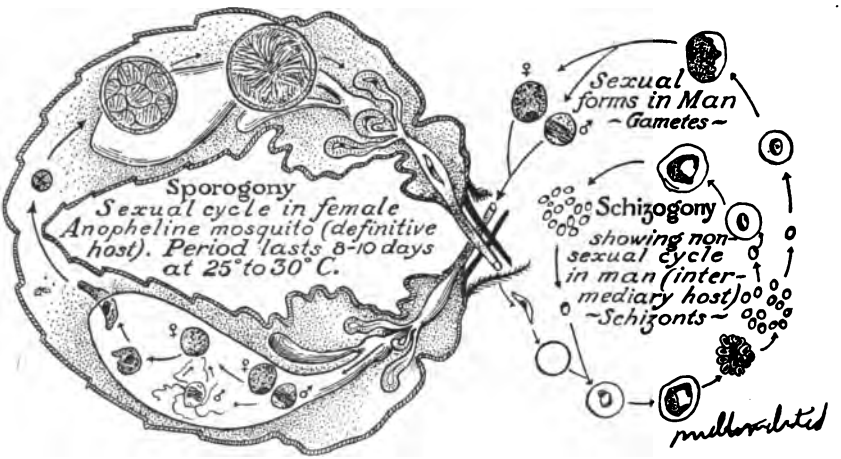


FIG. 72.—Sexual (sporogony in mosquito) and nonsexual (schizoongy in man) cycle of the malarial parasite. The sporogony diagram to the left shows in lower portion the fertilization of the female gamete by the microgamete. The vermiculus stage of the zygote is shown boring into the walls of the mosquito's stomach to late become the more mature zygote packed with sporozoites as shown in the upper diagram of the developmental processes in the mosquito's stomach.

the end of one week it has grown to be about 50μ in diameter and has become packed with hundreds of delicate falciform bodies. Some only contain a few hundred, others several thousand.

Zygotes.—In some of his observations Darling has noted that the zygote of benign tertian malaria grows larger and more rapidly than that of æstivo-autumnal and that the pigment is clumped rather than in belts or lines as with æstivo-autumnal. Darling has also noted that mosquitoes do not tend to become infected unless the gamete carrying man has more than 12 gametes to the cubic millimeter of blood.

The capsule of the mature zygote ruptures about the tenth day and the sporozoites are thrown off into the body cavity. They make their way to the salivary glands and thence, by way of the veneno-salivary duct, in the hypopharynx, they are introduced into the circulation of the person bitten by the mosquito, and start a nonsexual cycle. As the sexual life takes place in the mosquito, this insect in the

definitive host and man is only the intermediate host. The sexual cycle or *sporogony* in the mosquito takes about ten to twelve days.

Efficient Mosquito Hosts.—It must be remembered that only certain genera and species of Anophelinæ are known malaria transmitters; thus Stephens and Christophers, in dissecting 496 mosquitoes of the species *M. rossi*, did not find a single gland infected with sporozoites.

With *M. culicifacies*, however, 12 in 259 showed infection. A mosquito which is capable of carrying out the complete sporogonous cycle is an efficient host and in the case of malaria the mosquito is the definitive host (sexual life of parasite).

Malarial Index.—Mosquito dissection is one method of determining the endemicity of malaria or the *malarial index*. There are two other methods: 1. by noting the prevalence of enlarged spleens, and 2. by determining the number of inhabitants showing malarial parasites in the blood.

This index is best determined from children between two and ten years of age, as children under two show for a general average too high a proportion of parasites in the peripheral blood while those over ten years of age show too great an incidence of enlarged spleens.

Barber working in the Philippines with children from five to ten years of age obtained a spleen index of 13.3 and a parasitic index of 11.

As before stated there are three species of malarial parasites: 1. *Plasmodium vivax*, that of benign tertian—cycle, forty-eight hours; 2. *Plasmodium malariæ*, that of quartan—cycle, seventy-two hours; and 3. *Plasmodium falciparum*, that of æstivo-autumnal or malignant tertian—cycle of forty-eight hours.

Multiple Infections.—Variations in cycles may be produced by infected mosquitoes biting on successive nights, so that one crop will mature and sporulate twenty-four hours before the second. This would give a quotidian type of fever. In æstivo-autumnal infections anticipation and retardation in the sporulation cause a very protracted paroxysm, lasting eighteen to thirty-six hours; this tends to give a continued or remittent fever instead of the characteristic intermittent type.

Plasmodium Vivax.—In fresh, unstained preparations, taken at the time of the paroxysm or shortly afterward, the benign tertian schizont, or nonsexual parasite, is seen as a grayish white, round or oval body, whose outlines cannot be distinctly differentiated from the infected red cell. They are about one-fifth of the diameter of the red cell and are best picked up by noting their amœboid activity. In about eighteen hours fine pigment particles appear and make them more distinct. After twenty-four hours the lively motion of the pigment and the projection of pseudopod-like processes, in a pale and swollen red cell, makes their recognition very easy.

When about thirty to thirty-six hours old the amœboid movement ceases. Approaching the merocyte stage the pigment tends to clump into one or two pigment masses and one can recognize small, oval, highly refractile bodies within the sporulating parasite.

The gametes or sexual forms do not show amœboid movement, but the fully developed gamete, which is generally larger than the red cells, has abundant pigment, which is actively motile in the male gamete and nonmotile in the female. The male gamete is more refractile, is rarely larger than a red cell and shows yellow brown, short rod-like particles of pigment. About fifteen minutes after the making of a fresh preparation these male gametes throw out four to eight long, slender, lashing processes, which are about 15 to 20 microns long. These spermatozoon-like bodies now break off from the useless parent cell and with a serpent-like motion glide away in search of a female gamete, knocking the red cells about in their passage through the blood plasma.

The female gamete is larger than a red cell, is rather granular and has more abundant dark brown pigment than the male.

Stained Smears.—In dried smears, stained by some Romanowsky method, as that of Wright, Leishman or Giemsa, we note small oval blue rings, about one-fifth of the diameter of the infected yellowish pink erythrocyte. One side of the ring is distinctly broader than the rather fine opposite end, which seems to hold a round, yellowish brown dot, the chromatin dot, and has a resemblance to a signet ring. These small tertian rings of the nonsexual parasites (schizont) are seen about the time of the commencement of the sweating stage of the paroxysm. Two chromatin dots in the line of the ring are rare as is also true of more than one ring in a red cell.

When the parasite is about twenty-four hours old we note that it contains much pigment and has an amœboid or multiple figure of eight contour, is about three-fourths the size of a red cell and that the infected red cell is about one and one-half times as large as in the beginning and presents a washed-out appearance. It is an anæmic-looking cell. We also note, as characteristic of a benign tertian infection, reddish yellow dots in the pale red cell, which are known as Schüffner's dots. These, practically, are characteristic for benign tertian.

A few hours before the completion of its forty-eight-hour cycle the contained pigment begins to clump, the chromatin to divide and, finally, we have a sporulating parasite, in which the 16 to 20 small, round, bluish bodies, with chromatin dots, are irregularly distributed over the area of the merocyte.

The gametes, or sexual parasites, show a thicker blue ring and have the chromatin dot in the center of the ring. The pigmentation of the half-grown gametes is more marked than that of schizonts of equal size. The shape of the gametes is not amœboid, as is that of the twenty-four- to thirty-six-hour-old schizont, but round or oval. *The full-grown gametes have the pigment distributed and the chromatin in a single aggregation—just the opposite of nonsexual parasites.* The male gamete stains a light grayish blue and has a very large amount of chromatin, usually centrally placed. The female gamete stains a pure blue, has only about one-tenth as much chromatin as plasma, with the chromatin often placed at one side. The pigment of the female gamete is dark brown while that of the male is yellowish brown.

Plasmodium Malariæ.—In fresh preparations the young quartan schizont has only slight amœboid movement and, as development proceeds, the rather dark

brown, coarse pigment tends to arrange itself peripherally about the band-shaped or oval parasite.

The infected red cell shows but little change. At the end of seventy-two hours the rather regular daisy form of merocyte is more distinct than that of the benign tertian merocyte.

The distinctions between the male and female gametes are similar to those of the benign tertian gametes. In Romanowsky stained smears it is difficult to distinguish the young quartan schizont from the benign tertian one but, after twenty-four hours, the tendency of the quartan schizont to assume equatorial band forms across a red cell of normal size and staining characteristics and without Schüffner's dots makes the differentiation easy. In the fully developed sporulating parasite or merocyte the eight merozoites assume a regular distribution giving it a daisy appearance.

The gametes show practically the characteristics of the benign tertian ones but are smaller.

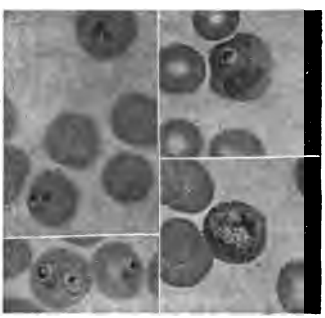


FIG. 73.—Tertian malarial parasites, one red cell showing malarial stippling. (*Todd.*)

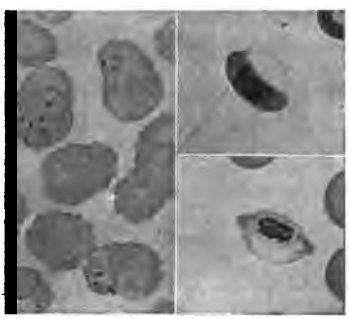


FIG. 74.—Æstivo-autumnal malarial parasites, and small ring forms and crescents. (*Todd.*)

Plasmodium Falciparum.—The young schizont of malignant tertian is extremely difficult to detect in fresh preparations, there being noted early in the rather long continued, hot stage, only small crater-like dots, about one-sixth of the diameter of a red cell which, however, show an active amœboid movement.

Later on in the hot stage these ring-like dots enlarge to become about one-third of the diameter of a red cell, most often occupying the periphery of the infected red cell. About this time, or at the very commencement of the pigmentation, the schizont containing red cells disappear from the peripheral circulation so that the further development is rarely observed in blood specimens.

The infected cell is brassy in color and shrunken in shape—it shows evidences of degeneration. The gametes appear as crescent-shaped bodies, which are absolutely characteristic of malignant tertian, the male gamete being more hyaline and delicate while the female one is more granular and larger.

In Romanowsky stained preparations we see, while the fever is sustained, small hair-like rings, with geometrical outline, with frequently two chromatin dots in one

end of the ring and a single red cell often showing two or more of these young rings. The rings are often seen as if plastered on the periphery of the red cells or as if having destroyed a rounded section of the rim of the red cell. As the fever declines the rings tend to disappear from the peripheral circulation. The infected red cells often show polychromatophilia and distortion.

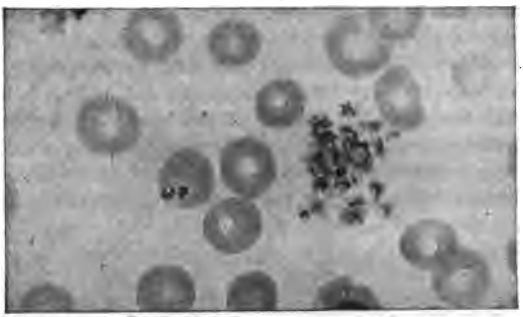


FIG. 75.—A cluster of blood-plaques and two plaques lying upon a red cell and simulating malarial parasites (×1000). (*Todd.*)

In old æstivo-autumnal cases, or those with severe infection, we may see adult rings and merocytes, which latter are smaller than those of benign tertian, show from 10 to 12 irregularly placed merozoites and a sharply clumped mass of pigment.

UNSTAINED SPECIMEN (FRESH BLOOD)

	P. vivax (benign tertian)	P. malariæ (quartan)	P. falciparum (malignant tertian) (æstivo-autumnal)
Character of the infected red cell.	Swollen and light in color after eighteen hours.	About the size and color of a normal red cell.	Tendency to distortion of red cell rather than crenation. Shriveled appearance. (Brassy color.)
Character of young schizont.	Indistinct amœboid outline. Hyaline. Rarely more than one in r.c. Active amœboid. movement. One-third diam. of r.c.	Distinct frosted glass disc. Very slight amœboid motion.	Small, distinctly round, crater-like dots not more than one-sixth diameter of red cell. Two to four parasites in one red cell common. Shows amœboid movement until appearance of pigment.
Character of mature schizont.	Amœboid outline. No amœboid movement.	Rather oval in shape. Sluggish movement of peripherally placed coarse black pigment.	Only seen in overwhelming infections. Have scanty fine black pigment clumped together.
Pigment.	Fine yellow-brown, rod-like granules which show active motion in one-half-grown schizont. Motion ceases in full-grown schizont.	Coarse almost black granules. Shows movement only in young to half-grown schizont.	Pigmented schizonts very rare in peripheral circulation except in overwhelming infections. Tends to clump as eccentric pigment masses almost black in color.

STAINED SPECIMEN

	P. vivax (benign tertian)	P. malariæ (quartan)	P. falciparum (malignant tertian) (æstivo-autumnal)
Character of infected red cell.	Larger and lighter pink than normal red cell. Shows "Schüffner's dots."	About normal size and staining.	Shows distortion and some polychromatophilia and stippling. Rarely we have coarse cleft-like reddish dots–Maurer's spots.
Character of young schizont.	Chromatin mass usually single and situated in line with the ring of the irregularly outlined blue parasite.	Rather thick round rings which soon tend to show as equatorial bands.	Very small sharp hair-like rings, with a chromatin mass protruding from the ring. Often appears on periphery of red cell as a curved blue line with prominent chromatin dot. Frequently two chromatin dots.
Character of half-grown schizont.	Vacuolated or Fig. 8 loop-like body with single chromatin aggregation. Schüffner's dots.	More marked band forms stretching across r.b.c.	Not often found in peripheral circulation. Chromatin still compact.
Character of mature schizont.	Fine pigment rather evenly distributed in irregularly outlined parasite.	Coarse pigment rather peripherally arranged in an oval parasite.	Very rarely seen in peripheral circulation in ordinary infection. Pigment clumps early.
Character of merocyte.	Irregular division into 15 or more spore-like chromatin dot segments. Mulberry.	Rather regular division into eight or 10 merozoites—Daisy.	Sporulation occurs in spleen, brain, etc. Rarely in peripheral circulation. Eight to 10 chromatin staining merozoites. (In culture 32.)
Character of macrogamete.	Round deep blue. Abundant, rather coarse pigment, chromatin at periphery.	Round, similar to P. vivax but smaller.	Crescentic, deep blue, pigment clumped at center, chromatin scanty and in center.
Character of microgametocyte.	Round, light green-blue, pigment less abundant, chromatin abundant and located centrally or in a band.	Round like P. vivax.	More sausage-shaped than crescent. Light blue. Pigment scattered throughout. Chromatin scattered and in greater quantity but difficult to stain.

The gametes are the striking crescent-shaped bodies and these show the distinctions of blue staining for the female, with lighter gray-green staining and abundance of chromatin for the male. The chromatin staining of crescents does not stand out so well as that of the round form gametes of benign tertian and quartan.

As regards differentiation of species and cycle the examination of stained smears is more satisfactory and definite, as well as less time consuming.

Still, one obtains many points of differentiation in the fresh preparation and should study such a preparation while carrying out the staining of his dried smear.

Blood platelets are the findings most frequently mistaken for malarial parasites in stained blood, and the vacuoles in fresh blood.

Central vacuolation of red cells is common in malarial anæmia and may be mistaken for nonpigmented parasites.

Malarial rings are usually peripheral and do not vary in size as one focuses up and down as do the central vacuoles.

A very puzzling but well-recognized finding in cases treated with quinine or salvarsan is the so-called quinine affected parasite. Such parasites lack definiteness of outline and show poor chromatin staining. The gametes do not seem to show these effects from the drug.

Cultivation.—As to cultivation of malarial parasites. Bass takes from 10 to 20 c.c. of blood from the malarial patient's vein in a centrifuge tube which contains $\frac{1}{10}$ c.c. of 50% glucose solution. A glass rod, or a piece of tubing extending to the bottom of the centrifuge tube is used to defibrinate the blood. After centrifugalizing there should be at least 1 inch of serum above the cell sediment. The parasites develop in the upper cell layer, about $\frac{1}{50}$ to $\frac{1}{20}$ inch from the top. All of the parasites contained in the deeper lying red cells die. To observe the development, red cells from this upper $\frac{1}{20}$-inch portion are drawn up with a capillary bulb pipette.

Should the cultivation of more than one generation be desired, the leukocyte upper layer must be carefully pipetted off, as the leukocytes immediately destroy the merozoites, Only the parasites within red cells escape phagocytosis. Sexual parasites are much more resistant. Bass thinks he observed parthenogenesis. The temperature should be from 40° to 41°C. and strict anaerobic conditions observed. Æstivo-autumnal organisms are more resistant than benign tertian ones. Dextrose seems to be an essential for the development of the parasites.

Bass considers that *P. vivax* has a disc-like structure which enables it to squeeze through the brain capillaries while adult schizonts of *P. falciparum* have a solid oval form which causes them to be caught in the capillaries.

The Thompsons have rather simplified the method of Bass. They draw 10 c.c. of blood into a test-tube containing the usual amount of glucose solution. They then defibrinate the blood by stirring with a thick wire for about five minutes and remove the wire with the adhering clot. They then pour this defibrinated blood into several small sterile test-tubes, which should contain at least a 1-inch column. Rubber caps are adjusted over the cotton plugs and the tubes placed in the incubator. They note the tendency of cultures of *P. falciparum* to agglutinate which is not true of *P. vivax*.

They think this agglutination the great cause of the plugging of capillaries in pernicious malaria. They note 32 merozoites as maximum number in sporulation of *P. falciparum* while *P. vivax* has usually 16 or more, but never as many as 32.

This would explain the shorter incubation period of malignant tertian. The pigment of *P. falciparum* clumps much earlier in the developing schizont than that of *P. vivax* and is much coarser and more discrete.

While Bass thought he noted parthenogenesis in cultures others have failed to observe any evidence of it.

PIROPLASMS

Belonging like the malarial parasite to the Hæmosporidia we have a group of parasites known as the PIROPLASMS. The correct name for these parasites in *Babesia* but they are better known under the name *Piroplasma*. They are minute organisms, usually pear or oval shape, which invade the red corpuscles. They produce no pigment but destroy the corpuscles and set free the Hb. which is excreted in enormous amounts by the kidneys. It is this which gives the name red-water to the disease usually designated Texas fever of cattle.

The cause of this disease is *B. bovis* (*B. bigemina*) and the parasite is transmitted by a tick, *Margaropus annulatus*. There is also a disease of dogs called malignant jaundice of dogs which is caused by *B. canis* and also transmitted by a tick. A disease of sheep, known as "Carceag," caused by *B. ovis* and transmitted by *Rhipicephalus bursa*, exists in the Balkan regions. Organisms of this kind have been thought of in connection with blackwater fever of man. Seidelin has claimed that a parasite of similar nature, *Paraplasma flavigenum*, was the cause of yellow fever.

At one time spotted fever of the Rocky Mountains was supposed to be due to a parasite named *Babesia hominis*. The parasite of Oroya fever is probably protozoal in nature. It is found in red cells as rod-like bodies and is called *Bartonella bacilliformis*. See Oroya fever. The parasite of Oroya fever has more of the characteristics of the genus *Theileria* than *Babesia*. In Africa there is a disease of cattle, called "Rhodesian fever," which is caused by a rod-shaped parasite in the red cells. The genus *Theileria* is characterized by bacilliform or coccoid shape rather than the pear-shaped one of *Babesia*. *T. parva* is the name of the parasite of Rhodesian fever.

SARCOSPORIDIA

Sarcosporidia are sporozoa found in the striped muscles of various mammals and birds. They are common in the pig and mouse and have been reported for man in three well-authenticated cases. In the last, Darling found these protozoa in the biceps muscle of a negro patient in Panama. In Baraban's case the laryngeal muscles at autopsy were found to show cysts about $\frac{1}{15}$ inch long which contained sickle-shape sporozoites about 9μ long.

They are known also as Miescher's tubes when in muscle fibers. These Miescher tubes or sarcocysts are elongated, round bodies which distend the muscle fibers. They vary greatly in length and thickness. These sarcocysts are filled with sickle- or oval-shaped spores, from 7 to 15μ long by 3 or 4μ wide. *S. muris* has been transmitted by feeding mice with the flesh of infected mice. It is thought that the spores

enter the epithelial cells of the intestines. They are divided into three genera *Miescheria* and *Sarcocystis* when parasitic in muscle fiber; *Balbiania*, when parasitic in the intervening connective tissue of the muscles. In some places more than 50% of the sheep and pigs may show infection.

Miescheria has a thin membrane surrounding the cyst while that of *Sarcocystis* is thickened and radially striated by small canaliculi. The present view is that we have only one genus of these parasites, viz., *Sarcocystis*.

As the young trophozoite grows nuclei increase and a definite membrane forms which the sporoblasts eventually fill. According to Minchin the Sarcosporidia contain only one genus, *Sarcocystis*. It is never parasitic for invertebrate hosts and while occasionally found in birds and reptiles it is preëminently a parasite of the higher vertebrates. As a rule, they are harmless parasites but the *Sarcocystis muris* is very pathogenic for the mouse. Closely related to the order Sarcosporidia is the parasite *Rhinosporidium kinealyi*.

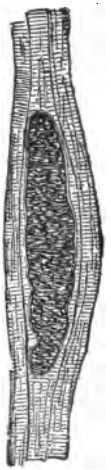


FIG. 76.—Miescher's sac from the musculature of a hog. ×30 diameters. (*After Ostertag.*)

Rhinosporidium kinealyi.—It causes pedunculated tumors of nasal cavity. The pansporoblasts enlarge in the center of the connective tissue of the nasal polyp and contain about 12 sporoblasts. When mature the cystic-like polyp bursts and the sporoblasts are liberated to extend the infection. When the tumor is incised it is seen to contain many white particles, about 250μ in diameter. These are the cysts which contain the pansporoblasts which are very small at the periphery of the cyst and quite large in the center of the cyst. Most of the cases have been reported from India.

CHLAMYDOZOA

These organisms are generally considered as being protozoal in nature and as a rule belong to the filterable viruses, which is the designation for the infectious principles of those diseases, in which filtration of defibrinated blood or serum through a Berkefeld filter capable of holding back so small an organism as the *M. melitensis*, does not prevent the infection being transmitted when introduced by the proper atrium of infection. The Chlamydozoa are also characterized by the occurrence of "cell inclusions."

The best known infections of this group of diseases in man are smallpox, vaccinia, rabies, trachoma, molluscum contagiosum, and foot and mouth disease. There are many such infections in other animals. The cell inclusions are regarded as products of cellular reaction to a virus which is more or less impossible of demonstration. The discovery of exceedingly minute granules in some of these diseases, as in variola and trachoma, has suggested that, as a reaction to the invasion by such a granule,

the cell throws an enveloping mantle about the invading particle. To designate this we use the name Chlamydozoa.

The generic name *Cytorrhyctes* has been applied to certain of these viruses, thus *C. vaccinia* develops within the epithelial cells of stratified epithelium. In vaccinia, Councilman and his colleagues consider that the development only takes place in the cytoplasm of the cell. In variola, however, the developmental cycle affects the nucleus.

Cytorrhyctes luis, reported as the cause of syphilis, sporulates in the blood-vessels and in the connective tissue, not in epithelial cells.

Cytorrhyctes scarlatinæ was reported by Mallory to have been found in the skin in four cases of scarlet fever.

CHAPTER XVII

FLAT WORMS

CLASSIFICATION OF THE PLATYHELMINTHES (FLAT WORMS)

Class	Family	Genus	Species
Trematoda	Fasciolidæ	Fasciola	F. hepatica
		Fascioletta	F. ilocana
		Fasciolopsis	F. buski
		Dicrocœlium	D. lanceatum
		Paragonimus	P. ~~westermanii~~
		Opisthorchis	O. felineus
		Clonorchis	C. sinensis
			C. endemicus
	Paramphistomidæ	Heterophyes	H. heterophyes
		Cladorchis	C. watsoni
		Gastrodiscus	G. hominis
	Schistosomidæ	Schistosoma	S. hæmatobium
			S. japonicum
			S. mansoni
Cestoda	Dibothriocephalidæ	Dibothriocephalus	D. latus
		Diplogonoporus	D. grandis
	Tæniidæ	Dipylidium	D. caninum
		Hymenolepis	H. nana
			H. diminuta
		Tænia	T. solium
			T. saginata
		Davainea	D. madagascariensis

NOTE.—Two larval Tæniidæ are found in man (*Cysticercus cellulosæ* and *Echinococcus polymorphus*).

Also two larval Dibothriocephalidæ (*Sparganum mansoni* and *Sparganum prolifer*.)

Two parasites often referred to as ophthalmic flukes have been reported lying between the crystalline lens and its membrane. They have been considered as possibly trematode larvæ. *Distomum ophthalmobium* was found in 1850 in the eye of a child and *Monostoma lentis* in the eye of an old woman.

TREMATODES OR FLUKES

Flukes are generally leaf-like in outline, rarely cylindrical, and exhibit marked variation in size and shape. They are nonsegmented and

possess a mouth and a pharynx. Very characteristic of them is the possession of suckers by which they hold on to the skin or alimentary system of their host.

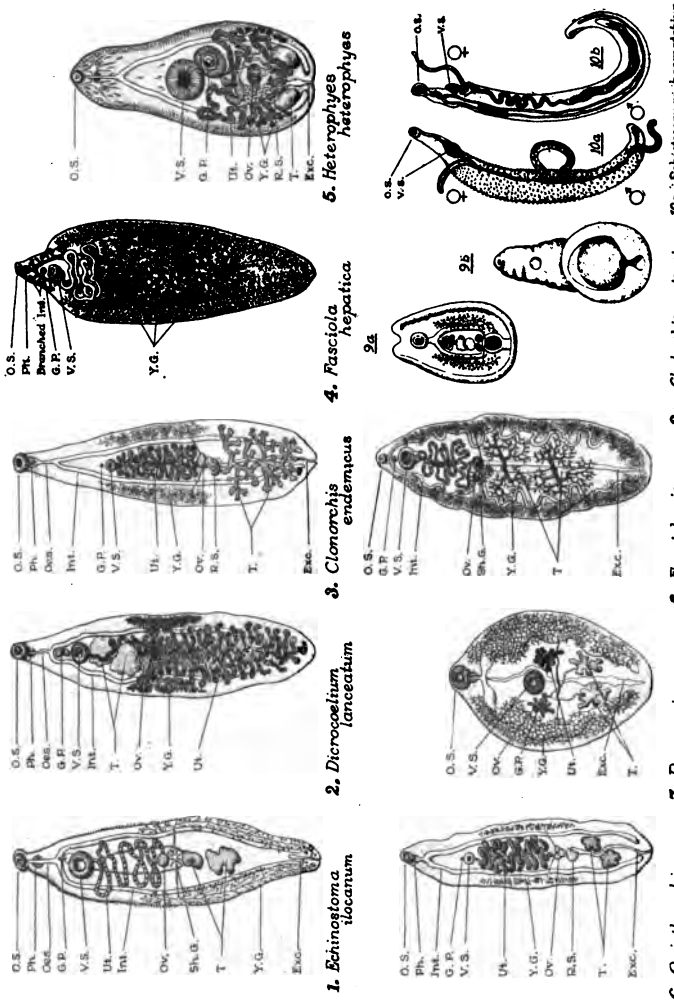


FIG. 77.—Anatomy of Trematoda (flukes) of man. *O.S.*, Œsophageal sucker; *Ph.*, pharynx; *Oes.*, œsophagus; *G.P.*, genital pore; *V.S.*, ventral sucker or acetabulum; *Ut.*, uterus; *Int.*, intestines; *Ov.*, ovary; *Sh. G.*, shell gland; *T.*, testicles; *Y.G.*, yolk glands or vitellaria; *Exc.*, excretory pore.

They are divided into two orders: 1. the Monogenea in which the egg gives rise to a larva which later becomes the adult and 2. the Digenea. It is to this latter that the flukes parasitic in man belong. This order is characterized by the fact that the larva becomes parasitic in some second animal and there gives rise to a second generation of larvæ which latter develop into adults.

The largest human fluke, *Faciolopsis buski*, is from 2 to 3 inches (50 to 75 mm.) in length, while the *Heterophyes heterophyes* is less than 1/12 inch (2 mm.) in length The most important fluke, the liver fluke, *Clonorchis endemicus*, is flat and almost transparent, while the almost equally important lung fluke, the *Paragonimus westermanii*, is oval, almost round and reddish brown in color. With the exception of the Schistosomidæ, all flukes are hermaphrodites, and, with the exception of this family, all flukes have operculated eggs. The only other operculated (with a lid) eggs we meet with in man are those of the Dibothriocephalidæ.

The three important families of flukes parasitic for man are:

1. Paramphistomidæ—flukes with two suckers situated at either extremity.

2. Fasciolidæ—flukes with two suckers, one terminal, the other adjacent to it and situated ventrally. This family includes the important genera *Fasciola*, *Opisthorchis*, *Dicrocœlium*, *Fasciolopsis*, and *Paragonimus*.

In *Paragonimus* and *Heterophyes* the genital pore is posterior to the acetabulum, in the other genera it is anterior. *Fasciola* has a dendritic intestinal canal which is not the case with *Clonorchis*, *Fasciolopsis*, *Fascioletta*, *Opisthorchis* and *Dicrocœlium*. In *Dicrocœlium* the testicles are anterior to the uterus, in *Opisthorchis*, *Clonorchis*, *Fasciolopsis* and *Fascioletta* they are posterior. *Fasciolopsis* and *Clonorchis* have branched testicles (the former a very large fluke-*Clonorchis* of medium size) while those of *Opisthorchis* are lobed.

3. Schistosomidæ: In this family we have a leaf-like male which by a folding in of its sides makes a channel for the thread-like female. The sexes are separate, not hermaphroditic as with the Fasciolidæ and Paramaphistomidæ.

Flukes have two suckers which, except in the Paramphistomidæ, are quite near each other—one is termed the oral sucker and the other the ventral sucker or acetabulum. The intestinal tract consists of a pharynx, proceeding from the oral sucker which bifurcates and terminates in blind intestinal cæca.

At the posterior extremity is an excretory pore which is at the termination of a duct which divides into ramifying branches. This is the water-vascular system. The testes, of various shapes and relations to the uterus, are more or less centrally situated and have vasa deferentia. In some flukes the receptaculum seminis is a conspicuous organ. The vitellaria are bilateral branching glands which pour nutrient material into the ootype. It is in the ootype that the eggs are formed, and opening into it we have the adjacent ovary. The shell gland surrounds the ootype.

A canal, known as Laurer's canal, leads from the ootype to the exterior, the function of which is in question. It is probable that as trematodes have no spermatheca, the spermatozoa from other flukes enter by way of this canal. The life history of many of the human flukes is now known. It is supposed that this, for others, might

resemble that of the common liver fluke of sheep (sheep rot). In this the eggs containing a ciliated embryo (miracidium) pass out in the fæces. This embryo is hatched out and, gaining the water, swims about actively until it reaches some suitable mollusk (*Limnæa truncatula*). By means of a pointed end, it bores its way into the body of the gastropod and in the pulmonary chamber becomes a bag-like structure (the sporocyst) from the germinal cells of which develop a creature with an alimentary canal (redia). The rediæ tend to break out of the sporocyst and wander to the liver of the snail. These rediæ may give rise to a second generation of rediæ.

From the rediæ minute little worms resembling adult flukes in possessing suckers, but differing in the possession of a tail, develop (cercaria). Having reached maturity, these cercariæ leave the rediæ, and, as in case of *Fasciola hepatica*, lose the tail, become encysted on blades of grass, to be eaten by sheep and again commence the cycle. The encysted cercariæ develop into adult liver flukes. It is probable that with many flukes the cercariæ enter some host, as mollusk, insect, or fish, and that it is by eating such animals as food that man becomes infected. Looss thinks it possible that the miracidium of *Schistosoma hæmatobium* may bore its way directly into man, as do the larvæ of the hookworm. Manson also suggests that the reporting by Musgrave of 100 mature lung flukes in a psoas abscess makes it very probable that these parasites entered the body as miracidia. It has recently been shown that certain molluscs form the intermediate hosts in schistosome infections, cercariæ from these molluscs entering the skin of man. With the lung fluke there would seem to be a primary mollusc intermediate host and a secondary one in certain crabs, which latter, when eaten raw bring about the infection. The idea in China is that the infection with the common liver fluke of man is brought about by eating fish. Fluke disease is generally known as distomatosis or distomiasis.

Liver Flukes

Fasciola hepatica (Distomum hepaticum).—This fluke, while of enormous economic importance by reason of destruction of sheep, has only been reported 28 times in man, and in these instances does not seem to have occasioned marked symptoms.

It has a cone-shaped anterior projection and is about 1¼ inches (30 mm.) long. The intestinal canal, as well as the testicles, is branched. These intestinal diverticula are well marked in the cone just after the branching from the œsophagus. Diameter of acetabulum about 1.6 mm., of oral sucker 1 mm. There is a possible importance of *F. hepatica* in connection with a peculiar affection known as "halzoun." This results from the eating of raw goat-liver, and it is supposed that the flukes crawl up from the stomach and, entering the larynx or attaching themselves about the glottis, produce the asphyxia characteristic of the disease. It is more probable that they attach themselves to the pharyngeal mucosa in the act of swallowing and do not enter the stomach where the gastric juice would destroy them.

Dicrocœlium lanceatum (D. dendriticum).—This has only been reported seven times in man. The symptoms are unimportant. The fluke is about ⅓ inch (8 mm.) long, with testicles anterior to the uterus.

Clonorchis endemicus (Opisthorchis sinensis).—This fluke and the *C. sinensis* are the most important of the human liver flukes. Until recently these flukes were known as *Opisthorchis sinensis.*

Looss has separated this genus from *Opisthorchis* principally by the characteristic of branching testicles—those of *Opisthorchis* being lobed. This fluke is very common in China and Japan—in certain sections of Japan 20% of the population being infected. This fluke is about ¼ to ½ inch (8 mm.) long and *C. sinensis* about ¾ inch long and 1/6 inch broad (16×4 mm.). When squeezed out of the thickened bile ducts it is so transparent and glairy as almost to resemble glairy mucus. As many as 4000 of these parasites have been found in a case, chiefly in the liver, but at times in the pancreas. This fluke is supposed to produce most serious symptoms, as indigestion, swelling and tenderness of liver, ascites, œdema, and a fatal cachexia. As a matter of fact, many physicians in China attribute very little pathogenic importance to it. The disease is diagnosed by the presence of the ova in the stools. The source of infection is probably through the eating of uncooked fish.

FIG. 78. — *Clonorchis sinensis.* (*Jefferys and Maxwell.*)

Kobayashi has examined various molluscs and fish for trematode larvæ. He succeeded in infecting nine kittens and two cats by feeding them with certain fresh-water fishes whose flesh contained trematode larvæ. These fish were found in districts where human distomiasis was common. More recently he has fed thirty cats on various cyprinoid fishes whose muscles contained encysted larvæ. In a series of dissections he has found that the ingested larval flukes have reached the bile ducts within fifteen hours and in twenty-six days they have reached maturity and begin giving off ova. He believes that the primary intermediate host is possibly a mollusc (*Melania libertina*) and that the fish is the secondary intermediate one. He does not consider that there are two species concerned in *Clonorchis* infections, as he has found variations in continuity of vitellaria in small as well as large flukes. Number of parasites present influences size. Age influences pigment production.

Looss considered the nonpathogenic C. *sinensis* to be larger (13–19 mm.), to show pigment in its parenchyma and to have breaks in the vitelline glands. C. *endemicus* was reported as smaller (10 to 13 mm.) and without pigment or breaks in the continuity of the vitellaria.

Opisthorchis felineus.—This fluke is smaller than the C. *endemicus*, and is a common parasite of the gall-bladder and bile ducts of cats. There are two-lobed testicles in this species instead of dendritic ones as in C. *endemicus*. In certain parts of Siberia the parasite is found in more than 6% of the human autopsies. The symptoms are similar to those caused by C. *endemicus*. The intermediate host of this fluke is in certain fish. These are eaten raw by natives.

Other liver flukes of less importance which have been reported for man are: 1 *Opisthorchis noverca.* This was found in bile ducts of two natives of Calcutta. It was lancet-shaped and covered with spines. It measures ⅓ by 1/10 of an inch (10×2.5 mm.). The eggs are 34 by 21μ.

2. ***Metorchis truncatus:*** This is a small fluke, 1/12 inch (2 mm.) long, squarely cut across at its posterior end and covered with spines. This was possibly found once in man.

Intestinal Flukes

Cladorchis watsoni (Amphistomum watsoni).—This fluke is about 1/3 inch (8 mm.) long, of oval outline but broader at posterior end and has an indistinct oral sucker and a large sucker at the other end. This parasite has been reported from northern Nigeria and is said to be a common infection of regions about Lake Chad. Eggs, 125×75μ. In the single reported case the parasites were passed in the stools. The symptoms were a watery diarrhœa.

Gastrodiscus hominis (Amphistomum hominis).—This fluke is about 1/4 inch (6 mm.) long and has a disc-like concavity, about 1/6 inch in diameter from which proceeds a teat-like projection, bearing an oral sucker. The acetabulum is in the posterior border of the disc. While it has only been reported a few times for man, indications are that it is probably fairly common in India and Assam. Eggs, 150×72μ. It gives rise to dysenteric symptoms.

Fasciolopsis buski (Distomum crassum).—This is probably a rather common parasite in India, as Dobson found the eggs in 1% of the stools of more than 1000 coolies. The fluke is from 2 to 3 inches (40 to 70 mm.) in length and about 1/2 inch (12 mm.) in breadth. It is thick, brown in color, and has a very large acetabulum, three times the size of the oral sucker and located almost adjacent to it. The branched ovary and shell gland lie in the center with the branched testicles posterior. The coiled uterus is anterior to the testicles. Eggs, 125×75μ. These parasites cause dyspeptic symptoms and an irregular diarrhœa. It is a rather common parasite in Cochin, China. It has been suspected that the larval stage may occur in shrimps. It is also called *Distomum crassum*. *F. rathouisi* is now considered to have been a shrunken *F. buski*, as it seems to be anatomically similar to *F. buski*. Kwan's fluke reported from Hong Kong, was possibly *F. buski*.

Heterophyes heterophyes (Cotylogonimus heterophyes).—This exceedingly small fluke (2×0.5 mm.), which can be recognized by its small size (less than 1/12 inch long) and large, prominent acetabulum, was formerly supposed to be rare. The oral sucker is much smaller than the acetabulum. The elliptical testicles lie at the extreme posterior end. Cuticle has scale-like spines. The eggs are 30×17μ. Very characteristic of this genus is the large sucker-like genital pore just below and to one side of the acetabulum. Looss has shown that it is quite common in Egypt, he having found it twice in Alexandria in nine autopsies. The parasites occupy the ileum. It is common in dogs.

Echinostoma ilocana (Fascioletta ilocana).—This is a small fluke, about 1/4 inch (6 mm.) long. There are two massive testicles in the posterior part of body. The acetabulum is prominent and about 500μ in diameter. This fluke has a ring of spines around the anterior extremity. Ovary anterior to testes. Genital pore anterior to acetabulum. The egg of this small fluke is quite large (100μ) and has an operculum. These trematodes were found by Garrison in five natives of Luzon, P. I., after treatment with male fern. Another intestinal fluke reported from Tamils in Malay States is *E. malayanum*. It is about 1/2 by 1/8 inch (12×3 mm.).

Metagonimus yokogawai.—This is a very small fluke, only about 1.5 mm. long, which burrows into the mucosa of the jejunum and causes a chronic intestinal catarrh. It occurs in Japan. The fluke is thickly studded with spines and has a peculiar deeply seated sac-like acetabulum.

Lung Flukes

Paragonimus ~~westermanii~~ (Distoma ringeri).—In certain parts of Japan and Formosa it is estimated that as many as 10% of the inhabitants may harbor this parasite.

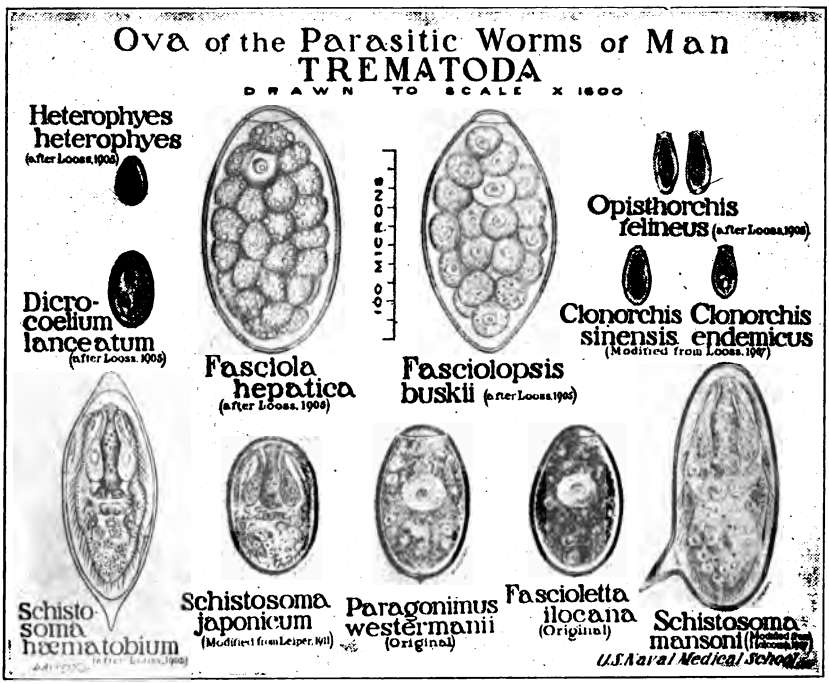


FIG. 79.—Trematode ova.

It is also common in China, and recently many cases have been reported in the Philippines.

It is popularly known as endemic hæmoptysis on account of the accompanying symptoms of chronic cough and expectoration of a rusty-brown sputum. After violent exertion, and at times without manifest reason, attacks of hæmoptysis of varying degrees of severity come on. The characteristic ova are constant in the sputum and establish the diagnosis. The fluke itself is a little more than ⅓ inch (8 mm.) long and is almost round on transverse section, there being, however,

some flattening of the ventral surface. The acetabulum is conspicuous and opens just anterior to the middle of the ventral surface. Eggs about 90×65μ.

The branched testicles are posterior to the laterally placed uterus and the genital pore opens below the acetabulum. The branched ovary is opposite the uterus on the other side.

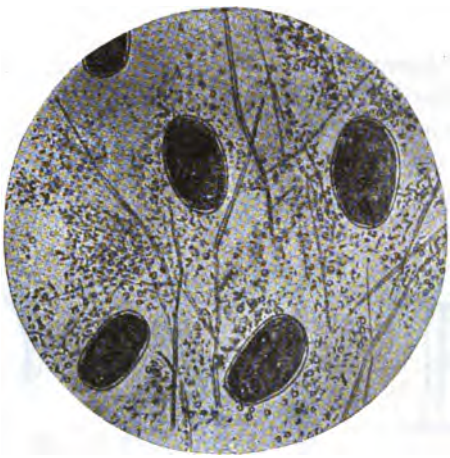


FIG. 80.—Sputum of man containing eggs of the lung fluke, greatly enlarged. (*After Manson.*)

It is rather flesh-like in appearance and is covered with scale-like spines. The flukes are usually found in tunnels in the lungs, the walls of which are of thickened connective tissue. There may be also cysts formed from the breaking down of adjacent tunnel walls. In addition to lung infection with this fluke, brain, liver, and intestinal infections may be found. Musgrave was the first one to call attention to the frequency of general infection with this parasite (paragonimiasis) in the Philippines. He found it in 17 cases in one year.

FIG. 81. — *Paragonimus westermanni:* natural size; to left showing ventral surface; to right showing dorsal surface. (*Braun after Katsurada.*) (*From Tyson.*)

Nakagawa has found that the miracidia infest certain fresh-water molluscs and become cercariæ in this first intermediate host. From this host the cercariæ go to certain fresh-water crabs and encyst in this second intermediate host, either in the liver or in the gills. In Japan one of these crab hosts, *Potamon dehaanii*, is eaten both raw and cooked.

Experimental feeding of puppies on infected crabs brought about infection with the lung fluke. It is thought that the fluke, after leaving the cyst, goes through the intestine to the abdominal cavity. Thence it perforates the diaphragm and enters the pleural cavity, finally penetrating the lung to become encysted there. The lung is the favorable site but wandering flukes may invade other tissues and organs even invading the central nervous system.

Besides man, dogs, cats and especially hogs may be infected.

Another fluke which has been reported from the lung is *Fasciola gigantea* (very similar to *F. hepatica*). This was coughed up by a French officer who had been in Africa.

Blood Flukes

Schistosoma hæmatobium.—Flukes of the circulatory system are of great importance in Egypt, South Africa, Japan, China, and the West Indies. The disease is named bilharziasis after Bilharz who in 1851 first associated the parasite and the disease.

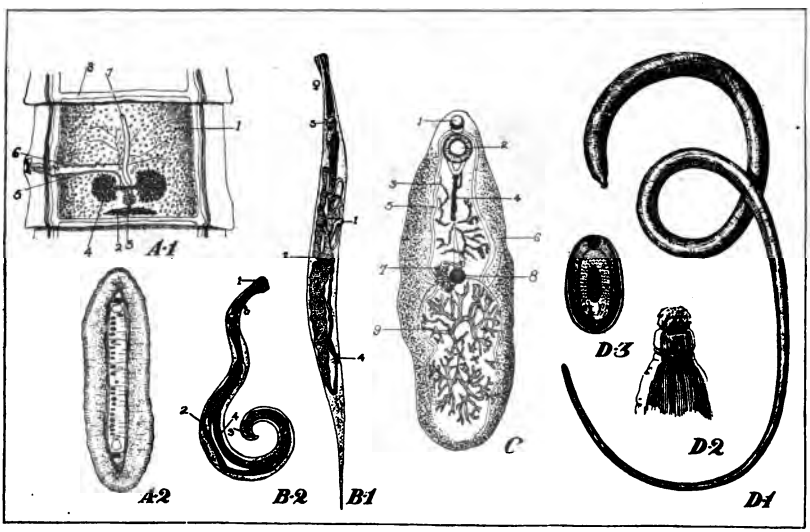


Fig. 82.—A 1. *Tænia saginata;* 1. Testicles; 2. Yolk glands; 3. Shell glands; 4. Ovaries; 5. Vagina; 6. Vas deferens; 7. Uterus; A2. Cross section of same. B 1. Female *Oxyuris;* 1. Vulva; 2. Ovary; 3. Bulb vesophagus; 4. Anus; B 2. Male *Oxyuris;* 1. Œsophageal inflation. C. *Fasciolopsis buski.* 1. Oral sucker; 2. Acetabulum; 3. Uterus; 4. Cirrus pouch; 5. Intestines; 6. Yolk glands; 7. Ovary; 8. Shell gland; 9, Testicles. D. 1, 2, 3, Worm, head and egg of *Echinorrhynchus gigas.*

It seems probable that there are at least three human species, differentiated principally by the appearance of the egg. In the blood-fluke, disease of Egypt (*S. hæmatobium*), the parasite chiefly infects the bladder and the egg has a terminal spine. The terminal-spined ovum is also found in the rectum and in the fæces. In the West Indies, as shown by the reports of Surgeon Holcomb from Porto Rico, rectal bilharziasis is rather common. In these cases the egg is prac-

tically always lateral-spined. Looss thinks that the lateral-spined egg is the product of an unfertilized female *S. hæmatobium.*

These flukes differ from other human flukes in possessing nonoperculated eggs as well as in having the sexes separate. The adults of this species, the *S. mansoni,* are scarcely, if at all, to be distinguished from the *S. hæmatobium.*

Leiper has recently noted a difference in that the male of *S. mansoni* has eight small testicles as against four large ones for *S. hæmatobium.* In *S. hæmatobium* the uterus is long, the ovary is in the posterior half of the body and the vitellaria in the posterior fourth of the body while for *S. mansoni* the uterus is quite short, the ovary is in the anterior half of the body and the vitellaria are in the posterior two-thirds of the body.

With *S. japonicum,* the name of the Eastern species, there is not only the difference that the eggs are without spines, but, in addition, the skin of the adult parasite is not tuberculated, as is the case with the other two species. It is slightly smaller, the acetabulum projects more prominently, and the lower part of the male infolds more markedly than in *S. hæmatobium.*

Catto considers that the *S. japonicum* may live in both arteries and veins. The other two species only live in branches of the portal vein. The blood flukes are about ½ inch (13 mm.) long. All of these flukes live separately until maturity. At this time the female enters what is known as the gynæcophoric canal of the male; this canal is formed by the infolding of the sides of the flat male fluke, thus giving a rounded appearance to the male. The female is longer than the male (about ⅝ inch long), and is thread-like and of a darker color. Her two extremities project from the canal of the male in which she lives.

The oral sucker of the male is infundibuliform and is smaller than the pedunculated acetabulum. In the female the oral sucker is larger than the acetabulum. The eggs of *S. hæmatobium* are fusiform, yellowish in color, have a thin shell and a terminal spine.

The most prominent symptoms of the Bilharz disease are hæmaturia and bladder irritation; later on calculus formation.

In rectal bilharziasis the symptoms are more those of bleeding piles or of a mild dysentery.

There may also be involvement of the appendix.

In the Japanese infection the symptoms point more to liver and spleen, there being ascites, cachexia, and a bloody diarrhœa. Early in the infection we have fever, urticarial spots and some bronchial

trouble (urticarial fever). The eggs should be searched for in the mucus cap on the fæces.

The eggs of the *S. japonicum* are readily found in the fæces; they are about 100 × 70μ. They are oval, transparent, and with a smooth shell, within which can be made out the outlines of an embryo. Upon adding water the ciliated embryo begins to show movement in about ten minutes and shortly afterward bursts out of the shell and swims about actively. It is more melon-shaped than the miracidium of *S. hæmatobium*.

Life History.—Katsurada, by experiments with a cat and dog, has proved that infection will take place through the shaved skin of an animal held in infected water —none of the water being allowed to enter by mouth. Fully developed miracidia and the male and female flukes were found in the portal vein. It is thought that further development of the miracidia in the body may account for the heavy infection.

Recently Leiper has found cercariæ showing the absence of a pharynx (characteristic of the genus) in a Japanese mollusc. Such molluscs were teased out in water and laboratory bred mice immersed therein. One of these mice was killed a month later and adult schistosomes were found in the portal vessels. Leiper has also found cercariæ showing absence of pharynx in four different species of molluscs in Egypt. With such molluscs he was able to infect white rats and other animals. He states that infection with these cercariæ from the mollusc host can bring about infection either by way of the mouth or through the skin. Sodium bisulphate in a strength of 1 to 1000 killed these cercariæ almost immediately.

It would therefore seem proven that all human schistosome infections take place following cercarial and not miracidial development. As proof that *S. hæmatobium* and *S. mansoni* are different species, Leiper notes that mice infected by molluscs of the genus *Bullinus* showed schistosomes with terminal spined eggs, the ovary lying in the lower half of the female. The male had four or five large testes. In mice infected by molluscs of the genus *Planorbis*, the eggs were lateral spined, the ovary was in the anterior half of the body and the male had eight small testicles.

Infection with the Bilharz parasites is brought about by bathing in water containing infected snails from which cercariæ have been thrown off from sporocysts of the snail, these cercariæ boring into the skin of the bather. Filtering the water removes these cercariæ. While gastric juice kills the cercariæ yet it is possible for them to penetrate the buccal mucosa and infect a person drinking an infected water.

CESTODE OR TAPE-WORM INFECTIONS

The cestodes and trematodes constitute the two great divisions of the flat worms. Anatomically, a tape-worm may be considered as a series of individual flukes united in one ribbon-like colony. The

cestode segments, or proglottides are covered by an elastic cuticle and in their interior usually contain striated elliptical bodies composed of calcium carbonate about 5 to 25μ according to the species in which they are found.

These calcareous bodies are characteristic of cestode tissue. They have been mistaken for coccidia. There is no mouth or alimentary canal in tape-worms, the segments absorbing their nourishment through the general surface.

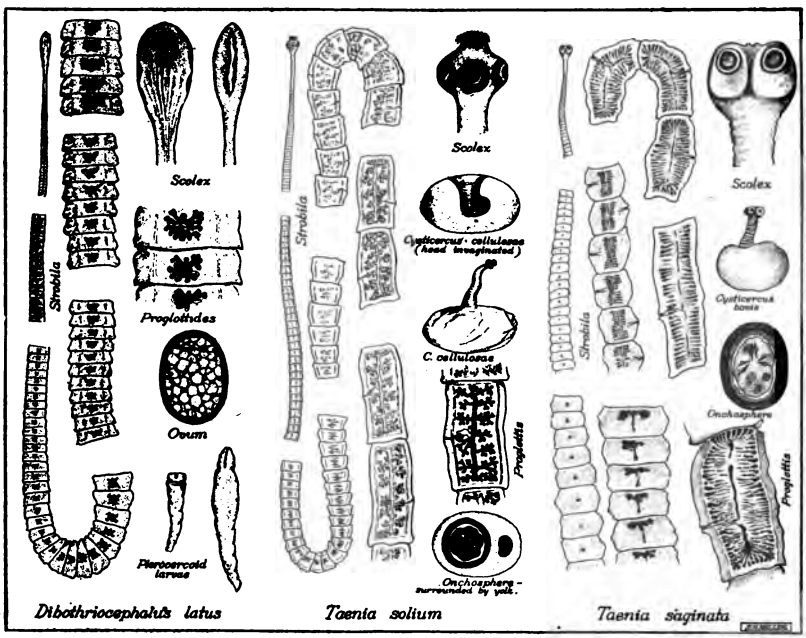


FIG. 83.—Adult and larval stages of cestoda of man.

A tape-worm is divided into the segment-producing controlling head and the series of segments or proglottides together known as the strobila. The head and neck together form the scolex.

The head contains the central nervous tissue and the commencement of the water-vascular system.

Tape-worm heads are provided with suctorial or hook-like organs, or both, to enable them to hold on to the intestinal mucosa.

The hooks when present on the anterior extremity of the head are carried by a protrusible structure called the rostellum.

The importance of the head is generally recognized by the well-known fact that the permanent evacuation of one of these parasites is only arrived at when the head as well as the segments is expelled. Otherwise, additional segments will be produced.

Even in tape-worms 25 to 30 feet in length, the head is no larger than a small shot. It carries the suckers or hooklets which best enable us to differentiate the different species. The segments adjacent to the head are immature—the sexually mature ones being found from the middle of the body onward.

T. saginata has about 2000 segments, *T. solium* less than 1000 while *T. echinococcus* has only three or four. The sexually mature segment possesses a varying number of testicles: three in *Hymenolepsis nana* and as many as 2000 in *Tænia saginata*. As with the flukes, they also have vasa deferentia, cirrus, ovaries, yolk glands, uterus, genital pore, etc. The location of the genital pore and the character of the branching of the uterus are of the greatest importance in differentiation. The sexually mature proglottides may either expel their ova, when these would be found in the fæces or, as is common, they break off and pass out themselves in the fæces. Then they either expel the eggs or may be eaten by some animal and in this way effect an entrance for their ova. It is an important practical point that the fæces of a patient with *T. solium* or *T. saginata* may not show any ova, these passing out in the intact segments. The oval operculated eggs of *Dibothriocephalus latus*, however, are constantly in the fæces.

The "hexacanth" or six-hooked embryo, also called the onchosphere, is the essential part of the egg. The embryonic envelope is dissolved off in the alimentary canal of the animal ingesting it, and the onchosphere bores its way through the gut to later become encysted in various tissues. In some tape-worms a ciliated embryo is liberated from the egg shell and swims about actively to enter some fish or other animal. When the six-hooked embryo reaches its proper tissue, the hooklets are discarded and a scolex similar to the parent one is developed. At this time we have a bladder-like structure with the scolex inverted in it. This is termed the proscolex stage. This little cyst with its scolex when ingested by another animal is digested, and the scolex, establishing itself in the intestine, develops a series of segments. The ciliated embryo of the *D. latus* does not form a cyst, but instead a worm-like creature similar to the adult. This is termed a plerocercoid.

If the larval stage shows a single cyst and a single head, it is termed *Cysticercus;* if multiple cysts but only one head to each cyst, *Cœnurus;* while with multiple cysts and multiple heads in each cyst the term *Echinococcus* is used.

Where there is very little fluid in the cyst and the larva is of minute size, as with the *Hymenolepsis*, the term *Cercocystis* is employed.

Key to Cestode Genera

I. Head with two elongated slit-like suckers—Genital pores ventral—Rosette uterus. Dibothriocephalidæ.

(*A*) Single set of genital organs in each segment. *Dibothriocephalus.*
(*B*) Double set of genital organs in each segment. *Diplogonoporus.*
(*C*) Immature forms showing characteristics of Dibothriocephalidæ—(collective group). *Sparganum.*

II. Head with four cup-like suckers; genital pores lateral. Tæniidæ.
(*A*) Uterus with median stem and a varying number of lateral branches. *Tænia.*
(*B*) Uterus without median stem and lateral branches.
(1) Genital pores single. Rostellum with not more than two rows of hooks.
(*a*) Suckers armed with numerous small hooklets. Fifteen to twenty testicles in each segment. *Davainea.*
(*b*) Suckers not armed. Three testicles in each segment. *Hymenolepsis.*
(2) Genital pores double. Rostellum with four or five rows of hooks. *Dipylidium.*

TÆNIIDÆ INFECTIONS

Tænia saginata (Tænia mediocanellata).—This very widely distributed tape-worm is often termed the unarmed tape-worm, to distinguish it from the *T. solium* or armed tape-worm.

It is from 10 to 25 feet long and has several hundred proglottides. The small pear-shaped head has four pigmented elliptical suckers and no hooklets. The segments are plumper than those of *T. solium*, hence the name saginata. The single lateral genital pore projects markedly and in a series of segments presents, as a rule, first on one side, and then on the opposite side of the next segment (alternating). The best way to distinguish a segment of the *T. saginata* from the *T. solium* is by counting the number of lateral uterine branches; these number fifteen to thirty, are quite delicate and branch dichotomously. The lateral divisions of the uterus of the *T. solium* are tree-like in their branching and only number five to twelve on each side.

T. solium has three ovaries while *T. saginata* has only two. The ox is the intermediate host of *T. saginata*. The eggs of *Tænia* have an oval outer shell which is filled with rather translucent, refractile yolk, often in globules. Within the oval shell is the more rounded cell of the six-hooked embryo with its thick striated membrane. The outer shell is often absent in the eggs found in the fæces, only the shell of the six-hooked embryo being found. The six-hooked embryo, having worked its way from the alimentary canal to the muscles or liver of the ox, becomes encysted (*Cysticercus bovis*). This little bladder-like structure is about ¼ by ⅓ inch, and contains but a small amount of fluid.

The evaginated head does not show hooklets, thus differing from the armed rostellum of the scolex of *Cysticercus cellulosæ.*

Being ingested by man's eating raw or imperfectly cooked meat, the adult stage becomes established in his alimentary canal in about two months.

It is probable that the various raw-meat cures have made the infection more common. *Cysticercus bovis* is more abundant in the tongue of cattle than elsewhere in the musculature.

For this reason it would seem advisable to use other raw meat than beef in such cures.

In Abyssinia the infection is said to be universal, and a man without a tape-worm to be a freak. An important point is the fact that the larval stage almost never appears in man. It is this fact which makes it a so much less dangerous parasite than the *T. solium*, which readily establishes a larval existence in man if the ova are introduced into the human stomach. Cooking meat always destroys the cysticercus. A period of about two months elapses after the ingestion of the cysticercus before the mature segments pass out of the rectum. These not only make their exit with the fæces, but are also capable of wandering out at other times. In this they differ from the segments of *T. solium*. *T. saginata* next to *Hymenolepis nana* is the common tape-worm of the United States. Dr. Stiles has examined several hundred tape-worms in the United States during the past few years and has found only one *T. solium*.

In Paris Blanchard found 1000 *T. saginata* to 21 *T. solium*. Certain German statistics, however, show about one-half as many *T. solium* as *T. saginata*.

Abnormalities of the scolex and proglottides are not uncommon with *T. saginata*. This is less frequently the case with *T. solium*.

Tænia solium.—The measly pork tape-worm is smaller than the *T. saginata* and differs from it in having a globular head, with a rostellum which is crowned by 26 to 28 hooklets.

In *T. saginata* a depression takes the place of the armed rostellum; the suckers of *T. saginata* are, however, much more powerful than those of *T. solium*. The segments have only five to ten coarse branches and are expelled only at the time of defecation. The segments or the ova having been ingested by a hog, the six-hooked embryo is liberated and becomes encysted chiefly in the tongue, neck, and shoulder muscles of the hog, as an invaginated scolex. Pork containing this cysticercus (*Cysticercus cellulosæ*) is known as measly pork. This cysticercus contains much more fluid than that of the ox and is from ¼ to ⅘ inch long. If one by chance should carry the egg on his fingers to his mouth, as the result of examining mature segments, the larval stage may be established in man. If this infection is not heavy, very few symptoms may be observed. The cysticercus, however, tends to invade the brain, next in frequency the eye, and so causes convulsions, death or blindness. Instead of only being the size of a pea, these cysts, when forming in the brain, may be the size of a walnut or larger. *T. solium* is comparatively common in north Germany, but is exceedingly rare in England and the United States.

Tænia africana.—This is an unarmed tape-worm, only about 5 feet long. It was found in a native soldier in German East Africa.

Garrison has reported from the Philippines a tape-worm with an unarmed rostellum, V-shaped and spiral formation of the uterine stem with compact structure of the gravid uterus under the name of *Tænia philippina*. Another tape-worm, *T. confusa* of which only segments were found was reported by Ward from Nebraska.

Hymenolepis nana (Tænia nana).—This is generally known as the dwarf tape-worm—it is the smallest of the human tape-worms. It is from ¼ inch to ½ inch in length, and is less than $\frac{1}{25}$ inch in breadth. (10×1 mm.).

The genus *Hymenolepis* has lateral genital pores, all of which are on the same side. These lateral genital pores cannot be made out in specimens as ordinarily examined. The head has four suckers and a rostellum, which is usually invaginated. The rostellum has a single row of 24 to 30 hooklets encircling it. Of the 150 to

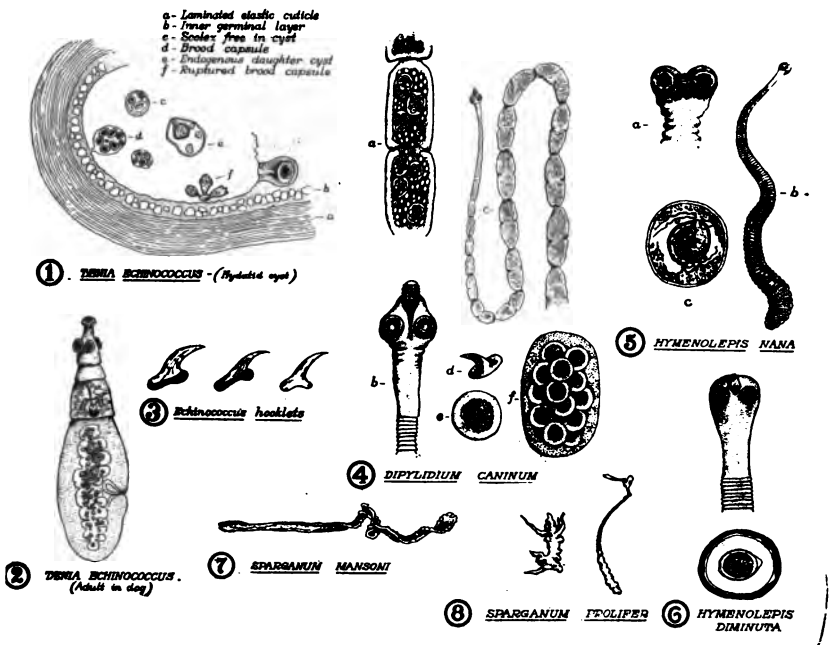


FIG. 84.—Other cestodes of man.

200 narrow segments the terminal ones are packed with eggs which in the last two or three seem to fill entirely the disintegrating segments. It would seem that the fully mature segments disintegrate and in this way the eggs are set free in the surrounding intestinal contents.

The worms as found in fresh fæces after tæniacide treatment are frequently in an advanced state of disintegration so that it is impossible to make out the head or hooklets.

The eggs of this species are quite characteristic, there being two distinct membranes. The inner one has two distinct knobs, from which thread-like filaments

proceed. The eggs of the *H. diminuta* have a thicker, striated, outer membrane and there are no filaments. The eggs of the *Dipylidium caninum* are similar, but are found in the fæces in aggregations—several eggs in a packet.

The dwarf tape-worm has been found to be the most common tape-worm in the United States. Dr. Stiles found it in about 5% of children in a Washington orphanage.

It has been estimated that in certain parts of Italy 10% of the children may be infected. The symptoms, especially nervous ones, may be marked in this infection. It has been incriminated as a cause of chyluria. Although very small, yet the number of parasites may be very great, even more than 1000. In a case that I treated with thymol there were 1500 worms expelled. A form found in rats, which may be identical with *H. nana*, does not require an intermediate host. This species, *H. murina*, has been found in the cysticercoid stage in the rat flea, so that an intermediate host may be required and it is possible there may be some other life cycle for *H. nana* than the following one which is usually given. The six-hooked embryo bores into the intestinal villus and there develops a *Cercocystis* (larva of small dimensions with but little fluid). When fully developed, it drops into the lumen of the gut, and a new parasite is added to the already existing number of parasites. This explains the heavy infection. *H. diminuta* and *H. lanceolata* have also been reported for man a few times.

H. diminuta is much larger than *H. nana*, being about 10 inches long. The suckers are small and the rostellum insignificant and unarmed. The intermediate host is some insect, as a moth; the definitive, the rat. As man is not liable to eat the insect hosts the infection is rare in man. Twelve cases have been reported for man of which five were from the U. S. It is now known that the rat flea acts as the intermediate host of this tape-worm.

H. lanceolata is common in geese and ducks. It has been reported as occurring once in a boy.

Dipylidium caninum (Tænia cucumerina) (T. flavopunctata).—This is a common parasite of dogs and cats. The larval stage is passed in lice and fleas. The cases of human infection have been principally in children, probably from getting dog lice or fleas in their mouths. The number of infections reported for man is about 40 and of these about 30 in children. The head has four suckers and a rostellum, which has three or four rows of encircling hooklets. The segments have the shape of melon seeds and have bilateral genital pores.

Davainea madagascariensis.—This tape-worm has been found in Siam and Mauritus. It is about 10 inches long. The head has four suckers and a rostellum with 90 hooklets. The suckers have rings of hooklets. The genital pores are unilateral. The cockroach is supposed to be the intermediate host.

There have been about 10 cases reported (Madagascar, Siam and British Guiana). There has also been reported a *D. asiatica*, the single specimen, however, lacking a head so that the exact genus is doubtful. It has been reported twice in children in Breslau. The intermediate host is thought to be a cyclops. Garrison reported cases from the Philippines.

DIBOTHRIOCEPHALIDÆ INFECTIONS

Dibothriocephalus latus (Bothriocephalus latus).—This is frequently termed the broad Russian tape-worm. It has a small olive-shaped head with two deep winding suctorial grooves on each side; it has neither rostellum nor hooklets.

The segments are quite broad, being about ½ by ⅕ inch. At the end of the strobila they are more nearly square. The segments are very numerous, 3000 or more. The fully developed worm is about 30 feet long. The uterus in each segment is rosette-shaped and the genital pore is ventrally situated. The eggs of this species have an operculum and a ciliated embryo. This ciliated embryo swims around and either enters some fish, especially pike, directly or through an as yet unknown intermediary. The embryo develops into a worm-like body with suctorial grooves at its anterior extremity and is found in the liver and spleen as well as in the muscles of the fish. This parasite produces an intense anæmia similar to pernicious anæmia. It is a frequent parasite in Switzerland, Bavaria, Japan, Scandinavia, and Russia. Recently several cases have been reported from our Northwest, and some of the fish of the waters of that region are said to be infected. The larva is a pleroceroid and is about 1 inch long. It is said that salting, smoking, or other ordinary methods of preserving fish will not kill it.

A tape-worm, *Diplogonoporus grandis* has been reported from Japan. In this there are two complete sets of genital organs to each segment.

FIG. 85.—Daughter cyst from hydatid cyst, considerably enlarged. (*Coplin.*)

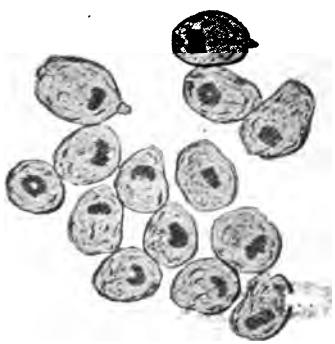


FIG. 86.—A group of daughter cysts from hydatid cysts. (*Coplin.*)

SOMATIC TÆNIASIS

While rarely we may have the larval stage of *T. solium* present in man, and while certain bothriocephalid larvæ (*Sparganum mansoni*

and *Sparganum proliferum*) infect man, yet they are unimportant as compared with the larval stage of the *Tænia echinococcus*.

Tænia echinococcus.—The adult stage of this parasite is passed in dogs. It is one of the smallest tape-worms known, being only about 1/6 inch long. It has a head with four suckers and a rostellum encircled with hooks. There are only three to four segments. The larval stage, on the contrary, gives one of the largest of larval cestodes. In man it may reach the size of a child's head.

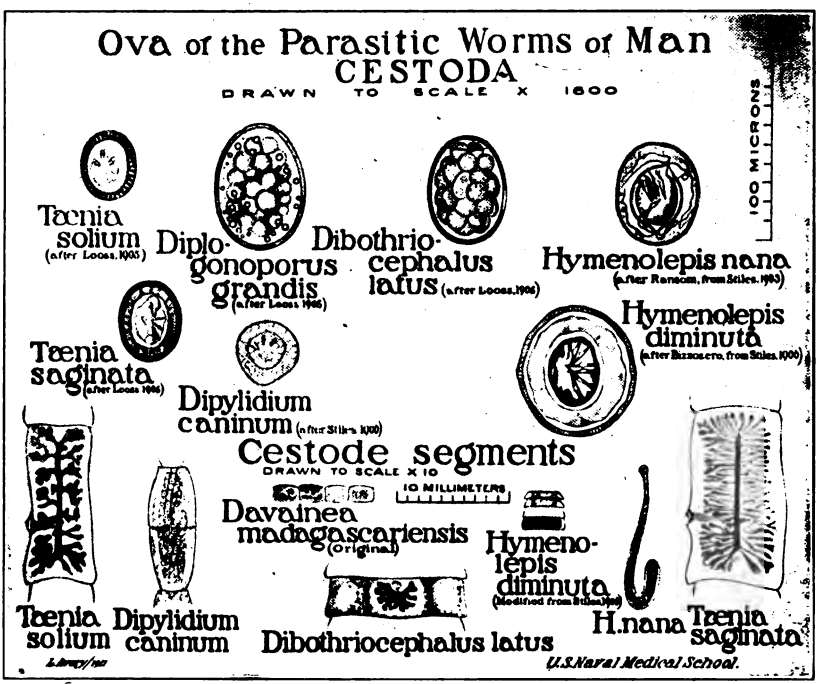


FIG. 87.—Cestode ova.

The larval stage is also found in hogs and sheep, and it is probable that by reason of the dog's eating the echinococcus cyst of such animals at the abattoir we owe the increase in this serious infection.

Man contracts the infection from association with dogs. The disease is peculiarly prevalent in Iceland, 1 in 43 inhabitants being infected. It is also quite common in Australia. As stated above, the adult stage is passed in the intestine of the dog. Should the egg-bearing segments passed by the dog contaminate the hands of man and a single egg be ingested, we may have hundreds of *Tænia* larvæ produced.

The six-hooked embryo, leaving its shell, bores its way through the walls of the alimentary tract and especially seeks the liver, just as the embryo of *T. solium* seeks the brain and eye.

Griffith notes that in Australia from 10 to 15% of hydatid cysts occur in the lungs. The cyst wall is quite thin and the hydatid cachexia seems to appear earlier in the lung than in the liver cases.

While probably 60% of cases have the cysts in the liver yet the kidneys or spleen may also contain them. Usually only one organ is invaded.

In the development of the cyst, after the embryo has come to rest at some point in the liver, we have formed at first an indistinctly laminated external envelope with coarsely granular fluid contents. Later on the contents become transparent, and two distinct layers can be observed: 1. The external, markedly laminated one, and 2. the internal one, made up of small cells externally and large cells and calcareous corpuscles internally. This internal lining membrane is known as the parenchymatous or germinal layer. When the external layer is incised it curls up by reason of its elasticity. This is characteristic of such a cyst. In addition, we have an enveloping connective-tissue capsule formed by the surrounding liver substance. From the germinal layer arise the brood capsules and the scolices. In these brood capsules we have the cellular layer external—just the reverse of the mother cyst. Scolices may develop either on the outside or inside of these brood capsules. It is interesting to note that one onchosphere may develop hundreds of scolices. From the parenchymatous layer of the mother cyst, daughter cysts are formed; these have an external stratified layer and an internal parenchymatous one; within them a varying number of scolices may develop. From these daughter cysts, granddaughter cysts may arise—all within the mother cyst—and hence are termed endogenous.

At times the daughter cysts work their way external to the mother cyst and proceed to develop in a manner similar to the endogenous formation. The exogenous development is rare in man, but common in hogs. Hydatids containing no scolices are called sterile. These cysts may be as large as a child's head, but are usually smaller. The fluid of these cysts contains about 1% of NaCl, also a trace of sugar; in addition there is a toxin which produces urticaria and acts as a cardiac depressant. If any quantity should escape into the peritoneal cavity at operation, it may cause death. Hydatids develop very slowly, and the duration of the disease is usually from two to eight years.

Echinococcus multilocularis is possibly due to a species different from *T. echinococcus*. In this we have a honeycomb arrangement with cavities filled with a gelatinous material. The majority of these cysts are without scolices. This form of hydatid is very fatal.

Sparganum mansoni (Bothriocephalus liguloides).—This is a larval bothriocephalid which is about 5 to 10 inches long and has been reported 10 times in Japan. It has been found in various parts of the body, as in pleural cavity, tissues about kidney, and in abscess of the thigh. They have been found in the urethra and under the conjunctiva. They resemble ribbon-like strings of fat. The anterior end is thickened and has two suctorial grooves. Yoshida recently fed a puppy with a larval

worm about 8 inches long and enclosed in capsule, which had been removed from the abdominal wall of a woman patient. The adult worm which developed in the dog resembled closely *D. latus*. The eggs of this species were much longer and narrower than those of *D. latus*.

Sparganum prolifer (Plerocercoides prolifer).—This has been reported from Japan as a larval form in the subcutaneous tissue. Stiles has found these larval forms in skin lesions in Florida. They show themselves as bizarre grub-like forms but may also appear as thread-like bodies. They reproduce by budding. When these buds are detached they give rise to a new worm so that instead of one worm in the subcutaneous cysts we may find several.

CHAPTER XVIII

THE ROUND WORMS

CLASSIFICATION OF THE NEMATHELMINTHES (ROUND WORMS)

Class	Family	Genus	Species
Nematoda	Angiostomidæ	Strongyloides	S. stercoralis.
		Dracunculus	D. medinensis
	Filariidæ	Filaria	F. bancrofti
			F. loa
			F. perstans
			F. demarquayi
			F. ozzardi
			F. philippinensis
		Onchocerca	O. volvulus
	Trichotrachelidæ	Trichuris	T. trichiura
		Trichinella	T. spiralis
	Strongylidæ	Eustrongylus	E. gigas
		Strongylus	S. apri
		Trichostrongylus	T. instabilis
		Triodontophorus	T. deminutus
		Œsophagostoma	O. brumpti
		Physaloptera	P. caucasica
		Ancylostoma	A. duodenale
		Necator	N. americanus
	Ascaridæ	Ascaris	A. lumbricoides
		Belascaris	B. mystax
		Oxyuris	O. vermicularis
Acanthocephali		Gigantorhynchus	G. gigas
Hirudinei		Hirudo	H. medicinalis
		Limnatis	L. nilotica
		Hæmadipsa	H. ceylonica

NOTE.—The *Strongyloides stercoralis* was formerly described under two designations: (1) *Anguillula intestinalis*, a parasitic generation and (2) *Anguillula stercoralis*, a free living generation.

ROUND WORMS OR NEMATODES

All nematodes are covered by a cuticle which varies in thickness, and is frequently ringed. The cuticle is moulted three or four times.

The cuticle is formed by the underlying ectoderm which is, as a rule, markedly developed in four ridges which divide the body into quadrants. Within the ectoderm is the body cavity, a space in which the reproductive organs lie in a clear fluid. The excretory system usually consists of two tubes which discharge near the head.

While the alimentary canal is more or less tube like in appearance it shows near the mouth a muscular œsophagus with a bulb-like expansion at the commencement of the remainder of the intestinal tract. There is a nerve ring around the œspohagus.

The testis and ovary are generally tube like. The sexes are, as a rule, separate. The male can usually be recognized by its smaller size, its curved or curled posterior end, and at times exhibiting an umbrella-like expansion—the copulatory bursa. The spicules, chitinous copulatory structures, may be observed drawn up in the worm or projected out of the cloaca. The genital opening of the female is ventral and usually about the mid-point; that of the male is close to the anus.

Certain papillæ in the region of the anus are valuable in differentiation. As a rule, nematodes develop in damp earth from the eggs as rhabditiform larvæ. Very few nematodes are viviparous (*Filaria*, *Trichinella*) being usually oviparous (*Ascaris*) less frequently ovoviviparous (*Oxyuris*).

The families Gnathostomidæ and Anguillulidæ are of very little importance in human parasitology. *Gnathostoma siamense* was once found in a breast tumor and *Rhabditis pellio* once in the urine.

Anguillula aceti, the vinegar eel, has been reported from the genito-urinary tract several times. Such cases can be explained by the prior contamination of the urine bottle or by the use on the part of the patient of a vinegar vaginal douche. The genera *Rhabditis* and *Anguillula* belong to the family Anguillulidæ.

A case of infection with a small nematode found in the papules of a skin infection, in a French boy is recorded as due to *Rhabditis niellyi*. The present view is that the parasites were embryos of *A. duodenale*, boring into the skin.

Angiostomidæ

In this family we have heterogenesis.

Strongyloides stercoralis.—This parasite was formerly thought to be the cause of Cochin-China diarrhœa. It presents two generations: 1. Parasitical or intestinal form. 2. The free living or fæcal form.

1. The intestinal form (also known as *Anguillula intestinalis*) is represented only by females. These are about $\frac{1}{12}$ inch (2 mm.) long and reproduce parthenogenetically. They have a pointed, four-lipped mouth, and a filariform œsophagus which extends along the anterior fourth of the body. The worm is so translucent

that it is difficult to detect it in the jejunal mucosa even with a hand lens. To examine for the worm scrape off the mucosa and search the preparation with a ⅔-inch objective. The anus is situated near the sharpened posterior end, the vulva about the lower third of the body. The uterus contains a row of eight to ten elliptical eggs which stand out prominently in the posterior part of the body by reason of being almost as wide as the parent worm. They usually live deep in the mucosa and the embryos emerge from the ova laid in the mucosa. The embryos escape from the eggs while still in the intestine, so that in the fæces we only find actively motile embryos. The eggs, which are strung out in a chain, never appear in the fæces except during purgation. As they greatly resemble hookworm eggs, this is a point of great practical importance. In fresh fæces we find hookworm eggs and *Strongyloides* embryos. The embryos are rather common in stools in the tropics. These embryos have pointed tails and are about 250×13μ. They have a double œsophageal bulb. They are about 250μ when they first emerge but may grow until they will approximate 500μ in the fæces. If the temperature is low, these rhabditiform embryos develop into filariform embryos, which being ingested form the infecting stage. It has been demonstrated that infection of man may also take place through the skin. If the temperature is warm, 25° to 35°C., these embryos develop into:

2. The free living form, *Anguillula stercoralis*. In this we have males and females, with double œsophageal bulbs, the male about 1/30 inch (¾ mm.) long with an incurved tail and two spicules and the female about 1/25 inch (1 mm.) long with an attenuated tail; these copulate and we have produced rhabditiform larvæ, which later change to filariform ones. At this time the length is about 550 microns. These, being ingested, start up the parasitical generation. If these do not reach the intestine they die out.

FIG. 88.—A, Egg of *Strongyloides intestinalis* (parasitic mother worm) found in stools of case of chronic diarrhœa; B, Rhabditiform larva of Strongyloides intestinalis from the stools. (*William Sidney Thayer, in Journal of Experimental Medicine.*)

FILARIIDÆ

This family is of the greatest importance to man. It is also one about

which much confusion exists as to the adult type; hence anyone finding adult filariæ should fix them in hot 5% glycerine alcohol (alcohol 70%), and subsequently mount in glycerine gelatin. Formalin is not to be used, other than for a very brief period (two to six hours) and then followed by the lacto-phenol method.

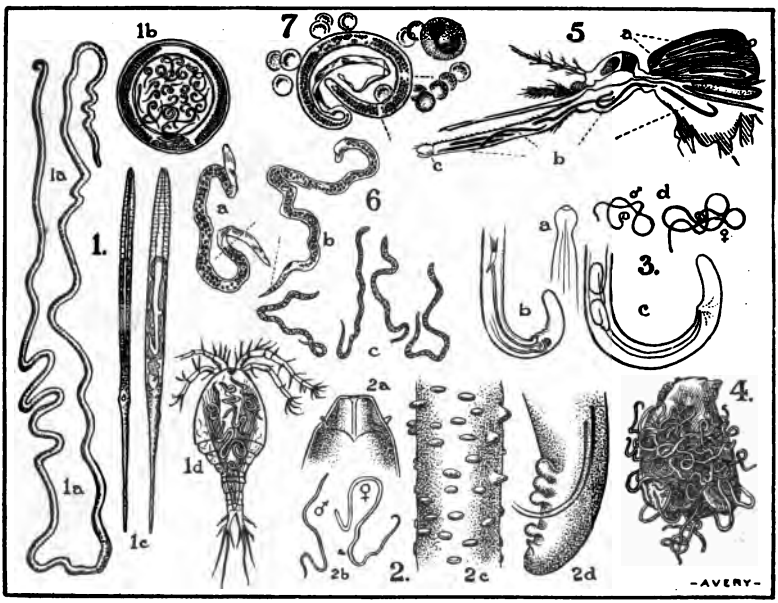


FIG. 89.—(1a) Adult female Guinea worm (*Dracunculus medinensis*) showing anchoring hook at posterior extremity. (1b) Cross section of female *Dracunculus* showing uterus filled with embryos. (1c) Striated embryos of the Guinea worm. (1d) *Cyclops coronatus*, the mintue crustacean which serves as the intermediate host of *D. medinensis*. (2a–2d) Anterior and posterior extremities of *F. loa*. (2c) Section showing tuberculated cuticle. (2b) Male and female *F. loa*, natural size. (3a) Bulbous anterior extremity, *Filaria bancrofti*. (3b) Tail of male. (3c) Tail of female. (3d) Male and female, natural size of *F. bancrofti*. (4a) Tumor mass of *F. volvulus* laid open. 5. Mosquito showing filarial embryos in thoracic muscles (a) and in labium (b). The labella which are separated from the labium by Dutton's membrane are seen at (c). 6. (a) Embryo of *F. bancrofti* (b) embryo of *F. loa* showing filling of tail end with cells. 7. Micrafilaria of *F. bancrofti* in blood. Dotted lines show location in break in cells column and V spot.

These worms are most likely to be seen as writhing thread-like worms, especially in the lymphatic glands and connective tissue, and about body cavities. They have a lipped or simple mouth and a filariform œsophagus. The male has an incurved tail with preanal and postanal papillæ which may be even corkscrew-like as in *F. immitis*. The spicules are unequal or there may be but one. The female is ovoviviparous, the vulva is at the anterior end and the uterus usually double.

Dracunculus medinensis (Filaria medinensis).—The Guinea or Medina worm, of which until recently only the female was known, is of great importance in parts of India, Africa, and Arabia. The female is a thread-like worm, about 20 to 30 inches long. The habitat is the subcutaneous and intermuscular connective tissue, especially of the lower extremity. It develops without symptoms. Finally a blister-like area appears on the surface of the leg, particularly about ankle-joint, which soon forms a painful ulcer. From this opening the anterior end of the worm projects to pour forth its striated embryos upon contact with water.

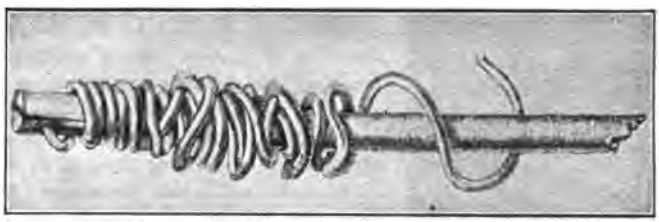


FIG. 90.—Guinea worm. Rolled on a stick for gradual extraction. (*From Greene's Medical Diagnosis.*)

The mouth is terminal and the body uniformly cylindrical. The uterus is a continuous tube filled with sharp-tailed, transversely striated embryos, 650×17μ, and constitutes the greater part of the body, the alimentary canal being pressed to one side. The genital organs probably discharge through the œsophagus. The body when being extracted is rather transparent. The tip of the tail is bent, forming a sort of anchoring hook. Recently Leiper fed monkeys on bananas containing infected *Cyclops*, and at the autopsy six months later obtained both male and female forms.

As regards the life history, Fedschenko, in 1870, showed that the embryos when liberated swam around in water and finally entered the bodies of species of the genus *Cyclops*. The female tends to come to the surface in the lower extremities, and experiments show that if on the blister-like points of emergence some water be squeezed out from a sponge, the uterus will eject a milky-looking fluid containing myriads of embryos. This would indicate that the worm selects the lower extremity so that the embryos may gain access to the *Cyclops* when the host is wading through the water. These larvæ enter the mouth of the *Cyclops* and after two moults become mature in about five weeks.

Leiper showed that a strength of HCl equal to that of gastric juice killed the *Cyclops*, but made the *Dracunculus* embryos very active. From this he judged that infection must probably take place from drinking water containing infected *Cyclops*. The suggestion of Leiper that wells harboring *Cyclops* be treated with steam, introduced by a pipe, seems to be valuable. The disease is known as "Dracontiasis."

Filaria loa (Filaria oculi).—This is a thread-like worm of west Africa about 1 to 2 inches long. The cuticle is characterized by distinct wart-like structures.

The anterior extremity is like a truncated cone with two papillæ at the base of the cone. The wart-like cuticular protuberances or bosses are about 12 to 15 microns in height. The females are 2 to 3 inches (50 to 70 mm.) long and about ½ mm. broad.

The males are smaller than the females and have three preanal papillæ and two postanal ones. There are two short unequal spicules. The life history is not satisfactorily established. The young are born ovoviviparously, and it has been suggested that the localized œdemas, known as Calabar swelling, may be due to the irritation produced by these eggs. These swellings are of hen's egg size, painless, do not pit on pressure and last about three days. They occur especially on the hands and arms. The embryos almost exactly resemble those of *F. bancrofti*. They have a diurnal periodicity, however, appearing in the blood about 8 A. M., increasing to noon and disappearing about 9 P. M. The adult worms have a tendency to wander about in the subcutaneous connective tissue, especially about the region of the orbit or even under the conjunctiva.

FIG. 91.—*Filaria loa* in the subcutaneous tissue, twice normal size. (*From Greene, after Fülleborn.*)

Adult worms of *F. loa* have been found and extracted, with an absence of the filarial embryos in the peripheral circulation of the patient. While immature adult worms have been extracted from children the embryos have only exceptionally been found in these children. This would speak for a very long developmental period for the adult worm and as a matter of fact the infection often only shows itself years after the opportunity for infection.

Leiper has just noted two species of *Chrysops* (Mangrove Flies) as intermediate hosts, the embryos developing in the salivary glands.

Filaria bancrofti (Filaria sanguinis hominis).—This is the most important of the filarial worms. It is a common infection in south China, India, the West Indies, and in the Pacific Islands, especially Samoa.

In medical books the embryos have been designated *Filaria sanguinis hominis*. This species is the cause of the common manifestations of filariasis, such as elephantiasis, varicose groin glands, chyluria, lymph scrotum, etc.

Filarial diseases are prone to lymphangitis attacks. Thus in lymph scrotum an erysipelatoid condition of the scrotum with high fever and chills may result. This condition is at times mistaken for malaria. Varicose groin glands may be mistaken for hernia. In the Philippines very few symptoms are noted in those affected with filariasis. Occasionally chylocele or chyluria and filarial abscess is reported.

F. bancrofti lives in lymphatics of trunk and extremities. At times the fine white thread-like worms may be seen as writhing coils in lymphatic glands.

The sexes are usually found together. The females are about 3 inches long and the males less than 2 inches. The tails of both sexes are incurved, but that of the male is more so. The head is club-shaped. The vulva opens 1.2 mm. from the anterior end. There are two uterine tubules. The sheathed embryos are supposed to be born viviparously and Manson supposes that as a result of injury to the parent worm and resulting extrusion of eggs, the blocking of lymph channels occurs

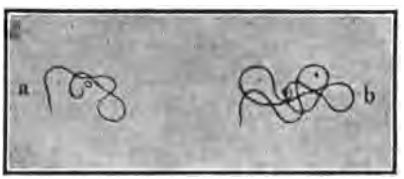


FIG. 92.—Male (a) and female (b) of *Filaria bancrofti*. Natural size. (*From Greene, after Manson.*)

A very interesting fact is that people with elephantiasis fail to show larvæ in the peripheral circulation. Manson considers that it is due to the blocking of the lymph channels.

These embryos show a nocturnal periodicity. During the day they remain in the lungs, and larger arteries.

If the patient sleeps in the daytime and is active at night the nocturnal periodicity or presence of embryos in peripheral circulation is inverted. In the case of *F. loa*, however, a change of habits does not change the periodicity of the filarial embryos, they continue to appear in the peripheral circulation by day even if the patient sleeps at that time.

The disease is transmitted especially by *Culex fatigans*. Many other mosquitoes can transmit the disease, anopeline as well as culicine ones. The sheathed embryos, getting into stomach of mosquito, wriggle out of the sheath, they then bore their way through walls of stomach and enter into a sort of passive stage, during which further development takes place. They finally become distributed in the muscles of the thorax and make their way along the fleshy labium, to enter the wound in a person bitten by a mosquito, by way of Dutton's membrane. This development takes about twenty days at which time the larvæ are about $\frac{1}{16}$ inch long and have an alimentary canal. It was formerly considered that the filarial worm of the Philippines was a different species, this from a study of microfilariæ (*F. philippinensis*). Others, however, have considered the microfilariæ as identical with *F. bancrofti*.

Recently Walker has published drawings and descriptions of four adult filariæ in the Philippines which correspond to *F. bancrofti.*

Filaria perstans.—The adults are found in connective tissue and deeper fat, especially about the mesentery and abdominal aorta.

The female is about 3 inches (75 mm.) long; the male is rarely found and is less than 2 inches long. These worms are characterized by incurved tails, the extremity of which has two triangular appendages giving a bifid appearance. The embryos do not possess a sheath and have a blunt tail. The life history is unknown. Both mosquito and tick have been incriminated. The embryos are always present in the pheripheral circulation—hence perstans. There does not seem to be any symptomatology.

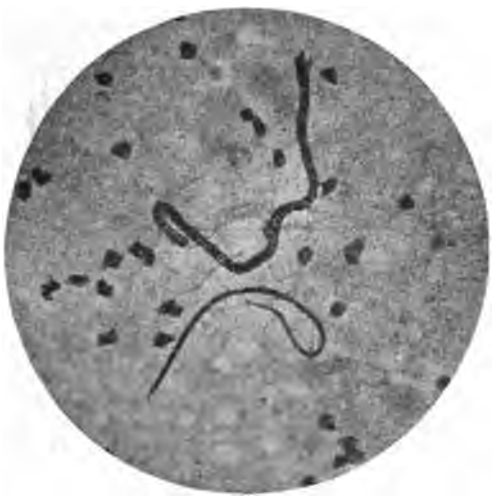


FIG. 93.—Filaria loa above; filaria perstans below. (*From Greene, after Fülleborn.*)

It is of historical interest that *F. perstans* was once considered the cause of sleeping sickness.

Onchocerca volvulus (Filaria volvulus).—This is a rather common parasite of central Africa. The male is about 1½ inches (35 mm.) and the female about 5 inches long. The females are so interlaced in the fibro-cystic swellings that it is difficult to determine their length. The tumors start from the presence of a worm in a lymphatic. The tumors are easily enucleated. Adults are striated. They are found in cystic tumors, especially about the axilla and popliteal space. The cystic contents contain abundant sheathless larvæ about 300μ long. It was formerly thought that these larvæ were absent from the peripheral circulation but more recent investigations have shown a sheathless embryo in the blood of patients with *Onchocerea* nodules which had the characteristics of those found in the contents of the nodules. Life history unknown. although it has been suggested that a species of *Glossina* may be concerned.

Filaria demarquayi.—The habitat of this filarial worm is the West Indies. The embryo has no sheath and has a sharp tail. Other filarial species which have been reported are *F. magalhæsi*, *F. ozzardi*, *F. volvulus*, *F. powelli*, and *F. philippinensis*. A species called *F. gigas* is now considered to have been only the hair of the leg of a fly. The embryos have usually been given such names as *F. nocturna*, *F. diurna*, etc. Of course the embryos and the parent should have the same name. It has been proposed to designate these embryos the same as the parent, but with the use of the term *Microfilaria* instead of *Filaria*.

The points usually noted in the description of filarial embryos are:

1. Presence or absence of periodicity of embryos in peripheral circulation.
2. Presence or absence of a sac sheath around the embryo.
3. Accurate measurements.
4. Shape and description of head and tail ends.
5. Character of movement.
6. Location of V spot and break in cell column in stained specimens.

Key to Filarial Larvæ in Peripheral Circulation

A. Sheath present.

1. No periodicity.
   F. philippinensis. Tightly fitting sheath; not flattened out beyond extremities. Tail is pointed and abruptly attenuated. Lashing progression movement. 320×6.5μ.
2. Periodicity exhibited.
   (*a*) Nocturnal periodicity.
   F. bancrofti (F. nocturna). Pointed tail; loose sheath; lashing movement. 300×7.5μ. V spot 90μ from head; break in cells 50μ from head.
   (*b*) Diurnal periodicity.
   F. loa (F. diurna). Pointed tail; loose sheath; 245 by 7 microns. V spot 60 to 70 microns from head, break in cells 40 microns from head.

B. Absence of sheath. None of these exhibit a periodicity, being continuously present.

1. Blunt tail—F. perstans. 200×4.5μ.
2. Sharply pointed tail:
   (*a*) F. demarquayi. 210×5μ.
   (*b*) F. ozzardi. 215×5μ.
   (*c*) O. volvulus. 250–300μ×7.5μ.

Note.—A filarial embryo, F. powelli, reported once. It has a sheath, nocturnal periodicity, and is about 130×5μ.

General Filarial Key

	Adults	Embryos	Remarks
Filaria bancrofti.	Male 40 by 0.1 mm. Female 90 by 0.28 mm. Smooth cuticle. Bulbous anterior extremity. Occupy lymphatic glands and vessels.	Sheathed, 300 by 7.5 microns. Distance from head to V spot 90 microns; to break in cells 50 microns. Tail rather straight. Terminal cells do not fill up tail end. Nocturnal periodicity in peripheral circulation.	Transmitted by mosquitoes. Culex fatigans and Stegomyia pseudoscutellaris. Causes elephantiasis, lymph scrotum; chyluria, etc.
Filaria loa.	Male 27 by 0.3 mm. Female 55 by 0.4 mm. Cuticle tuberculated. Anterior extremity like truncated cone. Wanders in subcutaneous tissues.	Sheathed, 240×7 microns. Distance from head to V spot 65 microns; to break in cells 40 microns. Corkscrew tail which is completely filled up with terminal cells. Diurnal periodicity in peripheral circulation.	Transmitted by species of a biting fly —Chrysops. Causes calabar swellings. Worms often visit ocular region.
Filaria perstans.	Male 40 by 0.07 mm. Female 75 by 0.1 mm. Cuticle smooth. Tip of tail shows two triangular processes. Found about root of mesentery.	Without sheaths, 200 by 5 microns. Posterior two-thirds tapers to blunt ending. Distance from head to V spot 49 microns; to break in cells 34 microns. Persists in circulation both day and night.	Transmitting agent not surely known. Mosquitoes and ticks suggested. No pathogenicity.
Filaria volvulus.	Male 30 by 0.14 mm. Female usually fragmented. Possibly 75 by 0.36 mm. Cuticle striated. Found coiled up in cyst-like tumors under skin.	Without sheaths. 250 by 7.5 microns. Found in cyst-like spaces of tumors. Found also in peripheral blood and lymph glands.	Method of transmission unknown. Causes small cystic tumors, under skin of thorax especially.
Filaria medinensis.	Male from Leiper's monkey 22 mm. Female 80 to 90 cm. long by 1.6 mm. wide. Smooth white body. Anchoring hook at tail end. Female lives in subcutaneous tissue of lower extremity.	Without sheaths. 600 × 20 microns. Long slender tail. Cuticle striated. Extruded from break in skin of patient.	Embryos swallowed by Cyclops. Man drinks water containing Cyclops.

Trichotrachelidæ

These have a long thin neck and a thicker terminal portion. The œsophagus is of the single row of cells type. The anus is terminal; there is only one ovary.

Trichuris trichiura (Trichocephalus dispar).—This is usually called the whip-worm—the thickened body representing the handle and the narrow neck the lash. It is one of the most common parasites in both temperate and tropical climates.

The egg is very characteristic in having an oval shape with knobs at either extremity. It resembles a platter with handles. The male is almost 2 inches long, and has the terminal portion curled up in a spiral. It has a single terminal spicule.

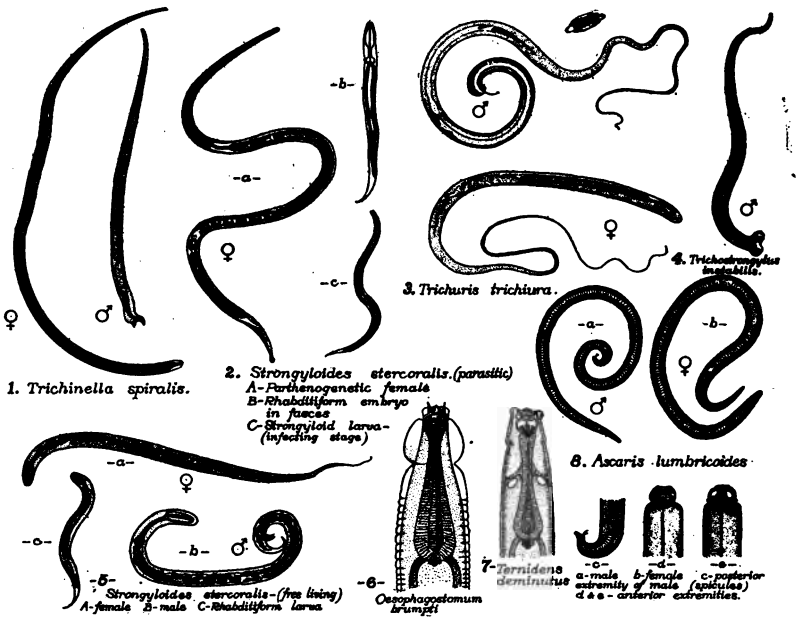


FIG. 94.—Some of the human nematodes.

The female is a little longer than the male, and has the terminal part in the shape of a comma instead of being coiled. The neck only contains the œsophagus which is contained in a groove in large cells which form a single row like a string of pearls. These cells play a digestion rôle. The vulva opens at the upper end of the thickened terminal end which contains an intestine lying between the ovary and uterus. The great powers of resistance of the ova may account for their general distribution; they may live for months under conditions of freezing and so forth. There is no intermediate host. The worm arrives at sexual maturity in about one month after ingestion. The whip-worm prefers the cæcum, but also lives in the lower end of the ileum and the appendix.

The neck burrows into the mucosa, and much importance has been attributed by the French to the possibility of this paving a way for the entrance of pathogenic bacteria. They do not seem to produce serious symptoms.

Trichinella spiralis (Trichina spiralis).—The cause of trichinosis is usually termed *Trichina spiralis* in medical works.

The adults live in the duodenum and jejunum; the males are about 1/16 inch (1.5 mm.) long with two tongue-like caudal appendages and without a spicule. These two lateral projections enable the male to hold the female in copulation—the cloaca being evaginated to act as a penis.

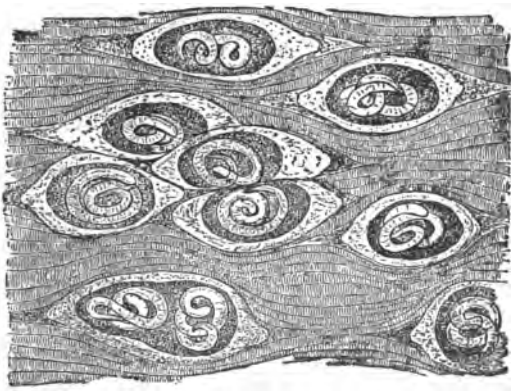


FIG. 95.—*Trichina spiralis.* (*Ziegler.*)

The females are about 1/7 inch (3 to 4 mm.) long. The female gives off embryos from the vulva which is near the mouth end (viviparous).

These parasites can be seen with an ordinary magnifying glass. With higher powers the œsophagus has the appearance of a serrated line instead of an œsophageal bulb. The male is about 40μ broad and has a prominent testicular enlargement filling the posterior extremity. The female is about 60μ broad and has a rounded posterior extremity with a prominent slit-like cloaca. It is in this posterior extremity that the female increases in size as she becomes filled with eggs. The vulva is in the anterior third. After fertilization of the females the males die, and the females bore into the intestinal mucosa and begin to produce embryos to the number of more than 1000 each. These gain access to the lymph channels and are distributed by the blood-stream to the striated muscles. Embryos reaching other tissues fail to develop.

It is about ten days before they reach the muscle. In the muscle they become encysted as the oval lemon-shaped areas containing coiled-up embryos that everyone is familiar with. These oval areas are about 450×250μ and have a chitinous capsule.

The encysted trichinæ are found chiefly in the muscle fibers of the tongue and diaphragm and may remain alive as long as ten to twenty years; finally, however, the cyst undergoes calcareous infiltration and the embryo dies.

When uncoiled the embryo is about 1 mm. long with the mouth at the attenuated end. Among cannibals it would be easy to keep the cycle going by eating improperly cooked or raw human meat, the parasite being thus transmitted.

As this would not explain the transmission among civilized men, the following is the life history: Man obtains his infection from eating raw pork, the embryos encysted in the muscle of the hog being liberated in the stomach, and the males and females developing in the intestine as above described.

The hog may gain his infection by eating the meat of other hogs or rats. These rats eat scraps of pork at slaughter houses and become infected. Being cannibals, rats when once infected, continue to propagate the infection.

In man, during the first two or three days while the adults are breeding in the intestine, we have gastrointestinal symptoms.

It is during this period or at any rate before the fifth day that purging may be of benefit. About ten or twenty days after infection the embryos begin to wander and we have the acute muscle pains. In the diagnosis we should try to obtain specimens of the pork which has caused the trouble in order to examine for encysted trichinæ, or to feed to white rats or rabbits, subsequently examining the diaphragm of these animals for encysted trichinæ or the intestine for adult trichinæ. Excision of a small piece of the deltoid of man may confirm the diagnosis. The best method is to take blood in 3% acetic acid, centrifuge, and examine for larvæ.

During the diarrhœal stage we may examine the stools for adult worms, in particular dead males or possibly actively motile embryos—these latter are about $90 \times 6\mu$.

Always examine the blood for eosinophilia.

It is well to remember that the parts of meat which trichinæ prefer (muscle of diaphragm, of neck, etc.) are often used in sausage. Unfortunately it is almost impossible to detect the embryos in sausage meat.

STRONGYLIDÆ

In this family the male has a caudal bursa, a prehensile sort of expansion at the posterior end for copulatory purposes.

The mouth is usually provided with six papillæ and at times with a chitinous armature. Those without the chitinous armature are included in the subfamily Strongylinæ (*Strongylus, Trichostrongylus*) while those having an armed mouth are in the subfamily Sclerostominæ (*Ancylostoma, Necator, Triodontophorus, Œsophagostoma, Physaloptera*).

Eustrongylus gigas (Dioctophyme gigas).—This is the largest round worm infecting man; it is usually found in the pelvis of the kidney (giant strongyle).

Two or more worms may so distend the kidney as to convert it into a mere shell. Pain, hæmaturia and other symptoms of pyelitis, together with the finding of the eggs, make the diagnosis. There seem to be seven authentic and eight doubtful cases of infection in man.

The females are about 40 inches (1 m.) long and about ⅓ inch (8 mm.) in breadth while the male is about 10 inches (25 cm.) long.

The collar-like copulatory bursa of the male distinguishes it from *Ascaris* as does also the dark red color. The source of infection is unknown but it has been suggested that the larval stage may exist in fish.

Many of the reported cases were simply fibrinous clots from ureters or wandering round worms.

The very characteristic ova, with gouged-out oval depressions, may be found in the urine, and are diagnostically confirmatory.

Strongylus apri (Metastrongylus apri).—This nematode is common in the lungs of hogs, producing a bronchitis in young animals but apparently harmless for adult ones. It has been reported once from the lungs of a six-year-old boy. The male is about 1 inch (25 mm.) long with two long spicules. The female is about 2 inches long and has a sharply hooked posterior extremity with the vulva just beyond the bend. The mouth has six lips. The eggs contain embryos when laid. It probably does not require an intermediate host.

Trichostrongylus instabilis.—This is a small strongyle formerly known as *Strongylus subtilis*. The male is about ⅙ inch (4 mm.) long, and the female about ¼ inch (6 mm.). Anteriorly it tapers to a pointed head end which is only about one-tenth the thickness of the posterior extremity. The male has a bursa and two prominent equal spicules. It has been found in the upper part of the small intestine of inhabitants of Egypt and Japan. It does not appear to produce symptoms. Ova like hookworm ones (73 to 90×42μ). Ransom gives *T. colubriformis* as the proper name. It is a common parasite of sheep and goats in the U. S. and may exist in man in such regions. Stiles has kept in mind the possibility of such infections in his Southern States hookworm work but has failed to find cases in man. The eggs are not only larger than hookworm ones but show later stages of segmentation.

Triodontophorus deminutus (Ternidens deminutus).—This is a small round worm with three forked teeth taking origin from the pharyngeal lobes. The collar-like mouth orifice is made up of 22 rounded plates just inside the round mouth opening. They are less than ½ inch long and have once been found in the intestinal canal.

Œsophagostoma brumpti.—Six young females were found in a cyst of the colon in an African negro. They were about ⅓ inch (8 mm.) long. The anterior end presents an ovoid protuberance with a second cuticular inflation just below it. This is termed the peristomic collar. The buccal capsule is very shallow and surrounded by about a dozen plates. The mouth has six papillæ.

Œs. stephanostomum.—This species has recently been reported by Thomas in a native of Brazil. It has 38 leaflets in the external crown. The large and small intestines of this case showed numerous cysts, each containing one worm.

Physaloptera caucasica.—Mouth with two equal laterally placed lips, each having three papillæ and three teeth. The male has a lancet-shaped posterior extremity and is about ⅛ inch long (14 mm. by 0.71 mm.). Female is about 1 inch long (27 mm.) with a rounded tail end. Found only once in the alimentary canal of a native in the Caucasus. Leiper has recently reported a species *P. mordens* from Uganda, one case. This species is about twice as large as *P. caucasica.*

The Hookworms of Man

The hookworm infections of man come almost entirely from two parasites, *Ancylostoma duodenale*, the Old World species, and *Necator americanus*, which is generally called the New World species from its having first been reported from the U. S. by Stiles. Hookworms belong to the class Nematoda and family Strongylidæ.

Quite recently Lane has reported a new species, *A. ceylanicum*, as having been obtained from three men in Bengal, after treatment. This species is the one that infects the civet cat in Ceylon. So far as we know the other human species belong solely to man.

The male hookworms are a little more than ⅓ inch (9 mm.) long and the females a little more than ½ inch (13 mm.) in length.

The males can readily be distinguished by their posterior, umbrella-like expansion or copulatory bursa. The tail of the female is pointed. The vulva of *A. duodenale* is located in lower half of the ventral surface; that of *N. americanus* in upper half.

The large, oval mouth of the Old World hookworm has four claw-like teeth on the ventral side of the buccal cavity and two knob-like teeth on the dorsal aspect. It also has a pair of ventral lancets below the four ventral teeth. One cannot make out a dorso-median tooth. In *N. americanus* the buccal capsule is round, smaller and the ventral teeth are replaced by chitinous plates. Dorsally there are two similar but only slightly developed lips or plates. A very prominent, conical dorso-median tooth projects into the buccal cavity. Through it passes the duct of the dorsal œsophageal gland. There are also four buccal lancets.

The copulatory bursa of the *Necator americanus* is also different, being terminally bipartite and deeply cleft in the division of the dorsal ray, rather than tripartite and shallow, as with *A. duodenale.*

The anterior extremity of *Ancylostoma* bends in the same direction as the general body curve while that of *Necator* hooks back in an opposite direction to the body curve. In general, *Ancylostoma* is larger and thicker than *Necator*.

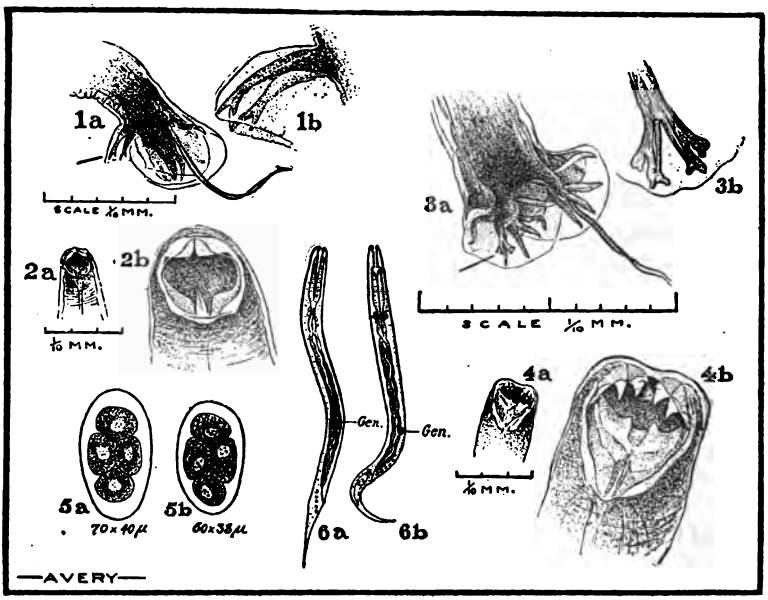


FIG. 96.—1a, Copulatory bursa of *Nectar americanus*, showing the deep cleft dividing the branches of the dorsal ray and the bipartite tips of the branches; also showing the fusion of the spicules to terminate in a single barb. Scale 1/10 mm. 1b, Branches of dorsal ray magnified. 2a, The buccal capsule of *N. americanus*. 2b, The same magnified. 3a, Cop. bursa of *Ancylostoma duodenale*, showing shallow clefts between branches of the dorsal ray and the tridigitate terminations. Spicules hair-like. 3b, The dorsal ray magnified. 4a, The buccal capsule of *A. duodenale*, showing the much larger mouth opening and the prominent hook-like ventral teeth. 4b, The same magnified. 5a, Egg of *N. americanus*. 5b, Egg of *A. duodenale*. 6a, Rhabditiform larva of *Strongyloides* as seen in fresh fæces. 6b, Rhabditiform larva of hookworm in fæces eight to twelve hours after passage of stool.

The name hookworm was given to these nematodes from the hook-like processes of the ribs of the rays of the copulatory bursa. Dubini called the Old World parasite *Agchylostoma*, properly *Ancylostoma*, on account of the four formidable hook- or claw-like ventral teeth of the buccal capsule. (αγχύλοσ, hook, and στομα, mouth.)

A. ceylanicum is somewhat smaller than *A. duodenale* and in the copulatory bursa of the male we have a deeper cleft in the dorsal ray and two rather long tips to each branch instead of the shallow cleft and three stumpy processes of the two branches as in *A. duodenale*.

Goeze found a hookworm in a badger in 1782. He named the parasite *Ascaris criniformis*. Froelich, in 1789, found hookworms in the fox and called them hookworms from the hook-like ribs of the copulatory bursa. He proposed the generic name *Uncinaria*. Therefore *Uncinaria* belongs to the hookworms of the fox and is not valid for any human species.

In 1838, Dubini found a hookworm as a human parasite. On account of the four ventral teeth projecting from the mouth he gave it the name *Agchylostoma* or correctly *Anyclostoma*.

Bilharz and Griesinger noted the connection of the parasite with Egyptian chlorosis, but it was not until the time of the St. Gothard tunnel (1880), that the importance of the parasite was recognized. Grassi noted the diagnostic value of

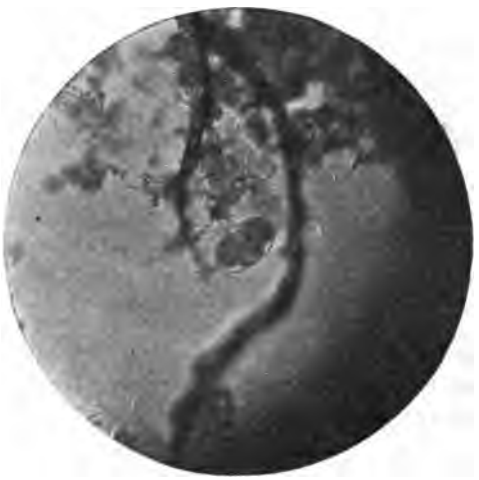


FIG. 97.—Ovum of *Ancylostoma duodenale*. By J. A. Thomson. (*Jefferys and Maxwell.*)

the ova in fæces in 1878. In 1902, Stiles noted and described the hookworm found in the United States as different and proposed the name *Uncinaria americana*, later changed to *Necator americanus*. A. J. Smith had also recognized the morphological differences.

Hookworms may be found in the small intestine (jejunum) of man in enormous numbers. They either produce their effects by feeding on the mucosa or by causing loss of blood.

Life History.—The delicate-shelled eggs pass out in the fæces, and in one or two days a rhabditiform embryo (200×14 microns) is produced. The mouth cavity of the embryo is about as deep as the diameter of the embryo at the posterior end of the mouth cavity; that of *Strongyloides* is only about one-half as deep as the diameter.

As a practical point, the anaerobic conditions in the intestines seem to prevent development of the hookworm ova or at any rate the absence of the oxygen, so necessary for the segmentations preliminary to the formation of the embryo, prevents it. Therefore hookworm ova in freshly passed fæces never show other than commencing segmentation while development of the larvæ of *Strongyloides* takes place in the intestines, so that in freshly passed fæces we find, generally, actively moving larvæ or at least eggs containing fully developed embryos. Hookworm ova very rarely show more than four segmentations or exceptionally eight in the freshly passed egg.

In the presence of oxygen these ova rapidly develop into larvæ, particularly at a temperature of about 27°C. Beyond 37°C. and below 14°C. development does not seem to take place.

The rhabditiform larvæ grow rapidly and by the third day are about 300 microns long and undergo a primary moulting. By the fifth day the bulb-like swellings disappear and the larva becomes possessed of a straight œsophagus, thereby becoming a strongyloid larva.

It then undergoes a second ecdysis or moulting, but instead of casting off this old covering, it retains it as a protecting sheath. At this time it ceases to take food but can move actively in its sheath so that it can crawl up blades of grass or vertical sides of mines. It is then about 0.6 mm. long. They can live in this stage for months, when moisture and shade are present, but are rapidly killed by drying.

This is the infecting stage in which the larvæ bore their way into the skin, which is the usual method of infection, or, occasionally, by entering the mouth on vegetables or otherwise.

Looss thought that they entered the skin by way of the hair follicles but the idea now is that they can bore into any part of the skin. It only requires a few minutes for the larvæ to enter the skin. From the subcutaneous tissues they effect an entrance into lymphatics or veins, go to the right heart, thence to lungs. From the alveolar capillaries they pass into the pulmonary alveoli, thence up the bronchi and trachea, to pass out of the larynx and then down the œsophagus to the stomach. The larva loses its protecting sheath in the stomach and in a few days develops a provisional buccal capsule.

By the end of the second week, after another ecdysis, the larvæ have grown to be about 2 mm. long and 130 microns broad and in about four weeks become adults, usually in the jejunum, where, after fertilization of the females by the males, the giving off of eggs begins. The adults attach themselves to the mucosa of the intestine, feeding on the deeper structures of the mucosa, or on the tissues of the submucosa. Sambon believes that the larvæ can work there way into the jejunum without going there by way of the trachea and œsophagus.

By providing an exit to the trachea, Fülleborn demonstrated that in dogs, infected with the dog hookworm, great numbers of larvæ poured out of the trachea. In other dogs he stitched the œsophagus to the skin and noted larvæ coming out of

these openings. In these dogs, with the ordinary channel obstructed, infection did occur with, however, only a few worms, thus showing the truth of Sambon's views but at the same time demonstrating the unimportance of such a route of infection.

The mouth cavity of the embryo is about as deep as the diameter of the embryo at the posterior end of the mouth cavity; that of *Strongyloides* is only about one-half as deep as the diameter. There is also a globular expansion at the bottom of the mouth cavity with hook-

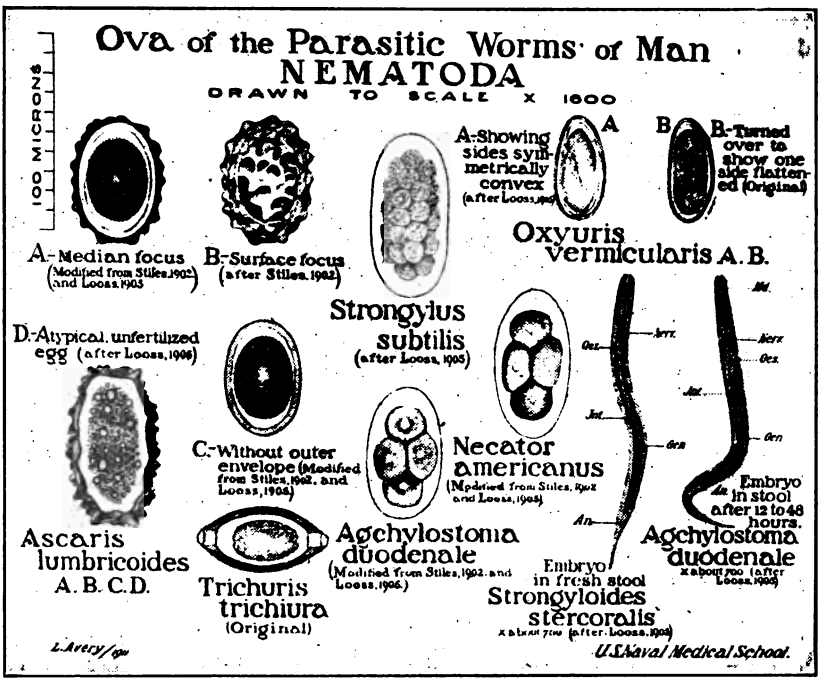


FIG. 98.—Nematode ova.

worm embryos while with *Strongyloides* ones the passage to the œsophagus is funnel-shaped. Also the genital anlage of *Strongyloides* is much larger than that of *Ancylostoma*, about 30μ long as against 4 or 5μ for *Ancylostoma*.

(A temperature of 1°C. kills the eggs in twenty-four to forty-eight hours.) After moulting twice, it remains rather quiescent but still lying inside the discarded skin. It reaches this stage in from four to fourteen days according to the temperature.

The soil in the area of the hookworm-egg-laden stool becomes infested with these larvæ which will even climb up blades of grass. It is for this reason that children with their bare feet are so liable to infection.

Laboratory Diagnosis.—As a matter of fact the diagnosis is almost invariably made by finding hookworm ova in the fæces. The eggs are oval and thin-shelled with a wide, clear, glassy zone separating the more or less segmented, granular central portion from the shell. Formed stools are more satisfactory for examination than the liquid ones resulting from a dose of salts. Put about 2 drops of water or 1% trikresol solution in the center of a glass slide and emulsify in it as much of the fæces as is held by the spatulate end of a wooden toothpick. A small piece of wood or a match stick will answer. These preparations can be readily examined without a cover-glass, using a ⅔-inch objective, with a 1-inch ocular.

It is usually stated that about 500 worms must be present for several months to produce symptoms. Grassi has thought that the presence of 150 eggs in 0.01 gm. fæces indicates the presence of 1000 worms, of which 25% would be males.

There may be as many as 4,000,000 eggs in a stool. Bass has proposed the following method for the examination of fæces for ova.

The fæces, which have been made fluid, should be centrifuged and the supernatant fluid containing vegetable débris poured off. The sediment contains hookworm eggs. Then pour on sediment a calcium chloride solution of sp. gr. 1.050. Again centrifuge and decant. Next add calcium chloride solution of a sp. gr. of 1.250 and centrifuge. This brings to the surface the hookworm eggs which may be pipetted off. As a rule, the finding of hookworm eggs is very easy without such a technic. Recently we have been using Barber's technic. Emulsify the fæces in equal parts of glycerine and saturated salt solution on a slide. The eggs rise to the surface and are easily picked up with the ⅔-inch objective. As a centrifuge method Barber emulsifies fæces in this same mixture which brings the eggs to the surface. A wisp of cotton is placed on the surface and 3 or 4 drops of melted agar dropped on the cotton. The disc of agar is removed with the cotton, deposited on a slide and examined for entangled eggs.

In certain cases, where a microscope is not available, the diagnosis may be made by finding the worms in the stool following a thymol treatment.

The presence of eosinophilia is of great assistance in diagnosis but it should be remembered that not rarely severe cases of the disease fail to show any excess of eosinophiles.

Charcot-Leyden crystals are often present in hookworm stools.

It has been claimed that where ordinary microscopical examination for ova will show 40% of infections and methods involving concentration 55% that cultural methods will show 99%. A convenient method of culturing is to make a pile of filter-paper circles of 2 inches diameter and about ¼ inch high and place in the center of a 4-inch Petri dish. Fill the dish with water about to the height of the filter-paper and spread a thick layer of fæces on the top of the filter-paper island. The larvæ hatch out in about six days and swim out into the clear surrounding water. They are best found by centrifuging the fluid containing them.

ASCARIDÆ

These have three papillæ or lips around the oral cavity, one dorsal and two ventral. The male has two equal-length spicules. An intermediary host is not needed in the life history of this family.

Ascaris lumbricoides.—The male round or eel worm is from 5 to 8 inches (18 cm.) long and the female from 7 to 15 inches (30 cm.) in length. They are grayish to reddish in color and are from 1/7 to 1/4 inch (5 mm.) in diameter.

It is probably the most common parasite of man, especially in children and as it does not require an intermediate host infection takes place through food or drink or by fingers of children who have been playing where soil pollution exists.

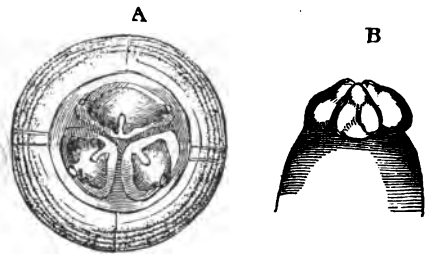


FIG. 99.—Anterior extremity of *Ascaris lumbricoides; A*, seen from front; *B*, seen from dorsal surface. (*Tyson after Railliet.*)

Stewart has recently noted that *Ascaris* eggs hatch out in the intestines of rats or mice and the larval worms then migrate to lungs, liver and spleen. After undergoing various developmental stages they leave the lungs go up the trachea and then down the œsophagus to the intestines. It was considered that these intestinal forms might pass out in the rat's fæces, contaminate food and infect man. Ransom notes the same development in a young pig fed *Ascaris* eggs as for mice, rats and guinea-pigs. Ransom is of the opinion that such a migration of larvæ occurs also in man and that lung symptoms may arise in infected children as for mice or rats and he does not believe any intermediate host necessary.

The normal habitat is the upper part of the small intestine, hence the ease with which they are vomited up. The three papillæ-like lips with a constriction just behind are easily studied with a hand glass. The very long, whitish, convoluted, thread-like tubes of the uterus lead to the opening of the vulva anteriorly and ventrally. The male has two large lance-like spicules which project from a subterminal cloaca. The posterior extremity of the male is curved ventrally and has seven pairs of postanal papillæ.

The body of the worm is transversely striated and resembles the ordinary earthworm, but is more grayish than red. The ova are very characteristic with a rough

mammillated exterior. This at times is shelled off and we have a smooth egg which may be mistaken for eggs of other parasites. The eggs leave the body in the fæces and after a long time—a few weeks to several months, according to temperature—develop an embryo which remains in the shell until swallowed by man. It is stated that they will remain alive for years. On being swallowed, the embryo leaves the egg and we have males and females developing in the small intestine. In countries where such parasites abound, as in Guam and the Philippines, the possibility of their getting into the peritoneal cavity through operative measures on the intestine must always be thought of.

Guiart considers it probable that *Ascaris* may suck blood, produce intestinal ulcerations and bacterial infections, and perforate intestine. Their entrance into bile ducts or into larynx (vomited) must be considered.

At autopsy they may be found perforating the appendix or even filling up the pancreatic duct.

Some think that the symptoms of itching of nose and anus, vertigo, or convulsions and anæmia may be due to a toxin secreted by the worm.

Belascaris mystax.—This is a very common parasite of the dog and cat, but is occasionally found in children. It is much smaller than the *A. lumbricoides*—male is 2 to 3 inches (5 cm.) long, female 4 to 5 inches (10 cm.) in length. The parasites are characterized by the presence of wing-like projections from the anterior end (arrow-like head). The egg shells are quite thin.

Leiper has reported an infection with *Toxascaris limbata* in an Egyptian. This is the smaller *Ascaris* of the dog and like the preceding species has cephalic wings.

Other Ascaridæ reported from man are *A. texana* and *A. maritima*, only one case each.

Oxyuris vermicularis.—The parasite is also known as the pin-worm or seat-worm and is more frequent in children than in adults.

The male is about 1/16 inch (4 mm.) long and the female a little less than 1/2 inch (12 mm.) in length. The male has an incurved tail with a single spicule and the female a long tapering tail. The vulva is in the upper third.

These worms have a clear slightly bulbous Turkish pipe mouth-piece-like projection surrounding the three-lipped anterior extremity. There is a well-marked bulb œsophagus.

The eggs are thin-shelled plano-convex, and show a coiled-up embryo. After ingestion of eggs, the adults develop in the small intestine where copulation takes place; the males then die. The fertilized females go to the cæcum and colon where they remain until they reach maturity. At this time the females wander to the rectum where they either expel their ova or themselves work their way out of the anus. This usually occurs at night, and the scratching induced by the itching causes the eggs to be widely spread about the region of the anus. The worms may also wander

into the vagina, urethra, or under prepuce. It will be seen that as a result of the scratching, the fingers become contaminated with ova which may be carried to the mouth and so cause a fresh infection, no intermediate host being required. The examination of the material under the finger nails of children harboring this parasite may show eggs under the microscope. A knowledge of the life history—the early location in the small intestine, and later on in the large—shows that treatment should be dual in its direction—enemata for the gravid female in the rectum and santonin and calomel for the young adults in the small intestine.

The diagnosis is preferably made by examining the stools for the white, thread-like females which are expelled after a diagnostic dose of calomel and salts, rather than by searching for the eggs.

These females, which are packed with embryo containing eggs, may be seen wriggling on the surface of the freshly passed fæces. In handling these worms one should be careful as they are apt to cause infection should the eggs get on the fingers.

ACANTHOCEPHALI

These are called thorn-headed worms on account of a proboscis which projects anteriorly like a little peg.

There are several rows of hooks surrounding this projection which are directed backward to enable the parasite to attach itself to the intestinal wall. The worm absorbs nourishment through the general body wall, there being no alimentary canal or mouth. These worms are common in hogs. The three-shelled eggs are very striking and the intermediate stage is in June bugs.

The Echinorhynchus or Gigantorhynchus gigas.—This parasite is about 6 inches (15 cm.) long for the male and 10 to 12 inches (25 cm.) for the female. It has transverse rings and resembles *Ascaris* but is more white in color. It is said to be not uncommon in southern Russia.

The Echinorhynchus or Gigantorhynchus moniliformis might be contracted by persons eating death-watch beetles as is sometimes done for the improvement of the complexion. The male is about 2 inches and the female about 4 inches long. A beetle, *Blaps mucronata,* is the usual intermediary.

HIRUDINEI (LEECHES)

Hirudo medicinalis.—This is the leech used medicinally for the abstraction of blood. They have a secretion which prevents coagulation of the blood so that when they are removed the wound still continues to bleed. These leeches are about 4 inches long and of a grayish-green color with dingy red stripes.

Limnatis nilotica.—This species has been found in many parts of northern Africa and, gaining access to the stomach through drinking water, it wanders to the pharynx, nares, and even trachea. Manson refers to a case of obstinate epistaxis and headache caused by a leech in the nostril.

This leech is about 4 inches long (8 to 10 cm.) and about ½ inch (1.2 cm.) broad. The dorsal surface is greenish brown with narrow orange-brown borders. The young leeches are only about ⅛ inch (3 mm.) long and taken in with the drinking water may attach themselves to the surface of some mucous membrane and after some weeks reach adult size.

Hæmadipsa ceylonica.—These are land leeches found in India, Philippines, Australia, and South America. They are only about 1 inch (25 mm.) long and are slender. They leave the damp earth to climb shrubs and from there to drop on animals or man passing through the forest. The bites are painless, but may be followed by ulcers. They may get into the nostrils.

They will even penetrate thick clothing in order to reach the skin.

CHAPTER XIX

THE ARACHNOIDS

Classification of the Arachnoidea

Order	Family	Subfamily	Genus	Species
Acarina	Trombidiidæ		Trombidium	T. holosericeum
	Gamasidæ		Dermanyssus	D. gallinæ
	Tyroglyphidæ		Tyroglyphus	T. farinæ
				T. longior
	Sarcoptidæ		Sarcoptes	S. scabiei
	Demodicidæ		Demodex	D. folliculorum
	Tarsonemidæ		Pediculoides	P. ventricosus
	Ixodidæ	Argasinæ	Argas	A. persicus
				A. miniatus
			Ornithodoros	O. savignyi
		Ixodinæ	Ixodes	I. ricinus
			Hyalomma	H. ægyptium
			Rhipicephalus	R. bursa
			Dermacentor	D. reticulatus
				D. andersoni
			Margaropus	M. annulatus
			Amblyomma	A. hebræum
			Hæmaphysalis	H. leachi
Pentastomida			Linguatula	L. rhinaria
			Porocephalus	P. constrictus

The class Arachnoidea and the class Insecta belong to the phylum Arthropoda. This phylum contains a greater number of species than does any other phylum.

While the lobsters, crabs and water fleas, which belong to the class Crustacea, are important zoologically, they are of very slight importance medically. Besides the Crustacea we have the thousand-legged worms or Myriapoda.

The different classes of Arthropoda resemble the segmented worms but have as distinction the possession of jointed appendages which proceed from the somites in pairs. Some of the pairs of limbs are for locomotion; at times, certain ones may be specialized for food taking.

The somites or divisions of the body have a chitinous exoskeleton. Respiration takes place through the medium of gills in the Crustacea and by tracheal tubes in the Myriapoda, Arachnoidea, and Insecta.

The Arachnoidea have no antennæ while the Myriapoda and Insecta have a single pair of antennæ, the former having numerous pairs of legs or jointed appendages while the latter have only three pairs of legs. The Arthropoda have segmented bodies, but they differ from the worms in having jointed appendages for the purpose of taking in food and moving from place to place. They also have an exoskeleton which is more or less unyielding from the deposit of chitin in the cuticle. This cuticle is not a true skin but only a secretion of the epidermis.

Within this external skeleton we have a dorsal digestive system and a ventral nervous system.

THE ARACHNOIDEA

The Arachnoidea differ from the Insecta in having the head and thorax fused together. They also have four pairs of ambulatory appenddages, while the insects only have three pairs. The Arachnoidea never have compound eyes—these when present being simple. Of the two orders of Arachnoidea of interest medically the Acarina is far more important than the Linguatulida.

Acarina

Of the acarines we are chiefly interested in the mites and the ticks. The acarines do not show any separation of the abdomen from the cephalo-thorax. A hexapod larva develops from the egg; this is succeeded by an octopod nymph which differs from the adult in not having sexual organs.

In addition to the four pairs of legs in the fully developed acarine there are two other paired appendages, the cheliceræ, in front of the mouth, and the pedipalps on either side of the mouth.

Trombidiidæ

These generally have a soft, more or less hairy integument and are often brightly colored. The two eyes are often pedunculated and the cheliceræ are lancet-shaped and the palps project beyond the rostrum as claw-like appendages. A tip-like appendage on the apical segment of the palps is characteristic. A very common and annoying member of this family is the hexapod larva of the *Trombidium holosericeum*. It is usually designated *Leptus autumnalis*. Popularly it is termed "harvest mite," "red bug" or "jigger." They are found in the fields in the autumn

and attack both man and animals. The condition (itching and redness) produced is at times called autumnal erythema. There is a *Trombidium* in Mexico which has a predilection for the skin of the eyelids, prepuce, and navel. The Kedani mite, an orange-red larval mite about 250 by 125 microns is believed by the Japanese authorities to bring about infection with Japanese river fever or Tsutsugamushi, as the result of transmitting either a bacterium or protozoon by its bite. The disease somewhat resembles typhus, although an eschar at the site of the bite and lymphatic involvement is present.

Gamasidæ

Of the Gamasidæ, which generally have a hard leathery body and styliform piercing chelicerae, delicate five jointed palps and styliform hypostome, only the *Dermanyssus gallinæ* is of interest. This mite infests chicken-houses and sucks the

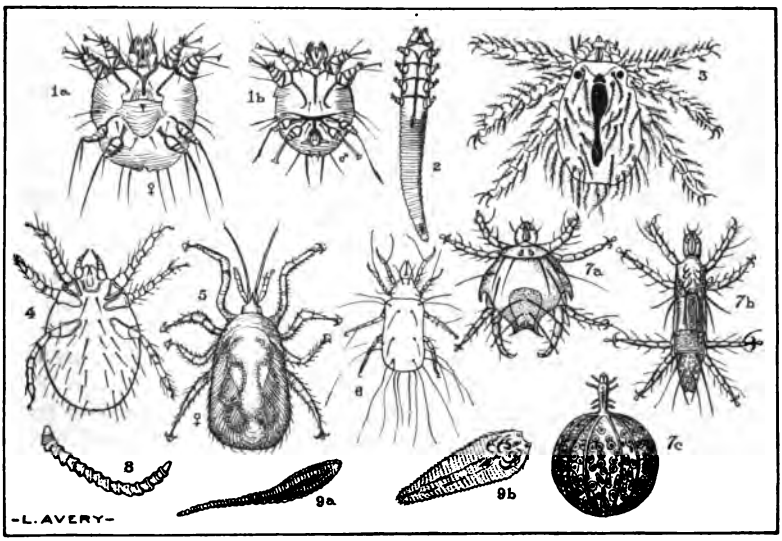


FIG. 100.—Arachnoidea exclusive of ticks. (1*a*) *Sarcoptes scabiei*, female; (1*b*) *S. scabiei*, male; (2) *Demodex folliculorum;* (3) *Trombidium akamushi*, hexapod larva (Kedani mite); (4) *Trombidium holosericeum* larva (*Leptus*); (5) *Dermanyssus gallinæ;* (6) *Tyroglyphus longior;* (7*a*) *Pediculoides ventricosus*, male; (7*b*) *P. ventricosus*, young male; (7*c*) *P. ventricosus* impregnated female; (8) *Porocephalus armillatus;* (9*a*) *Linguatula serrata*, female; (9*b*) *L. serrata*, larva.

blood of the inmates. They will also attack man. Poultrymen may be troubled with a sort of eczema on the backs of the hands and forearms, similar to scabies, resulting from bites by these mites. They measure 350×650μ. They have no eyes.

Tyroglyphidæ

Mites of this family live on cheese, flour, dried fruits, etc. They are small, without eyes, and have a smooth skin and a cone-like appearance of the mouth

parts which are largely formed by the chelate cheliceræ. They are chiefly of importance because of their being occasionally found in urine, fæces, etc., and being striking objects, the question of pathogenicity arises. The *T. longior* has been associated with intestinal trouble (probably a coincidence, patient having eaten cheese containing these mites).

Glyciphagi are found in sugar and are the cause of what is known as "grocers' itch." *Rhizoglyphus parasiticus* is reported to be the cause of an itch-like affection of the feet of coolies on tea plantations. To distinguish: the dorsum of *Glyciphagus* is hairy or plumose; *Tyroglyphus* has both claws and suckers on tarsi, while *Rhizoglyphus* has only claws.

Sarcoptidæ

These are small eyeless mites with a transversely striated cuticle. They live on the epidermis of man and various animals. The rostrum is chiefly made up of chelate cheliceræ with quite short three jointed, rather adherent palpi. It is the female that makes the tunnels in the skin between the fingers, on penis, flexor surface of forearm, etc. The male dies off after copulation. The females passes through four stages: 1. larva; 2. nymph; resembles adult, but has no sexual organs; 3. the pubescent female; 4. the egg-bearing female. A pair of itch mites may produce 1,500,000 descendants in three months. Transference of eggs, larvæ or pubescent females does not seem to transmit scabies. It is the egg-laden female only. The human itch mite, *Sarcoptes scabiei*, is an oval mite, the male is $250 \times 150\mu$; the female is about $400 \times 300\mu$. Besides the difference in size, the male may be distinguished from the female by the fact that the third and fourth pairs of legs in the female have bristles, but in the male, the fourth pair has suckers. The tunnels made by the female have the egg-bearing female at the blind end; scattered all along are fæces, egg, larvæ; the eggs being next the mother and the more mature young at the entrance to the gallery. A diagnosis can be made from the finding of either eggs or larvæ. The eggs are 140μ long and hatch out in four to five days. A female becomes mature in about two weeks.

In treating itch with sulphur preparations the adult females and immature itch mites are killed; the eggs, however, are not affected. Hence a second treatment about ten days after the first is necessary to kill the young mites, which have developed subsequent to the first treatment. Different animals have different species of itch mites.

Demodicidæ (Hair Follicle Mites)

Demodex folliculorum.—This is a vermiform acarine about 400μ long; the eggs are about 75μ long; they chiefly live in the sebaceous glands of nose and forehead.

Tarsonemidæ

This acarine family shows a complete sexual dimorphism. The last two pairs of legs are widely separated from the front legs. The *Pediculoides ventricosus* is oval and about $125 \times 75\mu$ for the male which has claws at the extremities of the

anterior and posterior pairs of legs; the two other pairs have hooklets and a sucking disc. The female is about twice as long but of the same breadth as the male, and has claws only on the anterior legs.

The cheliceræ are needle like with inconspicuous palps and the front and rear pairs of legs are widely separated. The gravid female is like a ball and is about 1000μ in diameter.

They live on wheat and may be found in wheat straw, which, if handled, may be followed by a severe skin eruption with an irregular fever.

Ixodidæ

This family of the Arachnoidea is one of great medical interest and of growing importance. It has recently been proposed to raise the ticks to a superfamily, Ixodoidea and to divide it into the families Argasidæ and Ixodidæ.

While only proven the intermediary hosts in the case of the organism of African tick fever and the recently discovered cause of spotted fever of the Rocky Mountains, there is considerable speculation as to the possibility of blackwater fever being due to a *Babesia* (*Piroplasma*). Piroplasmata of animals seem to be invariably transmitted by ticks.

Very important diseases due to these small pear-shaped organisms within red cells are known for various animals, the best known being that of cattle in Texas and known as Texas fever. Other piroplasmata diseases are Rhodesian fever (cattle), heart water (sheep), and malignant jaundice of dogs. In these diseases there are pathological features which resemble blackwater fever of man.

It is of interest to note that it was with the transmission of Texas fever through an intermediate host (the tick) that Smith and Kilborne (1889–1893) established the zoological principle of transmission of disease through arthropod intermediary hosts. This led up to the work on malaria, yellow fever, etc.

Ticks differ from insects in having four pairs of legs, only two pairs of mouth parts, and no antennæ. They differ from other acarines in having a median probe-shaped puncturing organ, the hypostome, which is beset with numerous teeth projecting backward, and in possessing stigmal plates. The head, or capitulum, or rostrum, is the part which projects anteriorly from the body. This carries the piercing parts which are the single hypostome or dart and a pair of piercing chitinous structures, the cheliceræ which lie above the hypostome. As a sheath for these delicate biting parts we have a segmented pair of palpi or pedipalps. The mouth is a slit between the cheliceræ and hypostome.

Two depressed pitted areas on the dorsal surface of the capitulum in the adult female are known as porose areas. Very important structures are the stigmal plates. These are striking mosaic-like areas which are located just posterior to

each hind leg in the Ixodinæ and between the third and fourth legs in the Argasinæ. As the greatest confusion exists as to the classification of ticks, Dr. Charles W. Stiles has now in hand a system of classifying ticks according to the appearance of these plates as seen under the high power of a microscope. There is great variation in the outline and general picture of these stigmal plates in the different species. The stigmal orifice, the opening of the tracheal system, is in the center. The Ixodinæ have a scutum or shield-like chitinous structure on the dorsal surface. It covers almost the entire back of the tick in the male and only a small portion anteriorly in the female. The genital opening is toward the anterior part of the ventral surface. The anus, with anterior or posterior anal grooves, is near the posterior third of the venter. The legs have six segments, the coxa being flattened out on the surface of the body and the terminal tarsus ending with a pair of hooks and at times with a pulvillus. The nymph has stigmal plates but has no genital opening while the larva has neither genital apertures nor stigmal orifice.

Life History of Ticks.—This varies greatly according to the subfamily, genus, and species. The female *Ornithodoros savignyi* lays about 140 eggs. The larva does not leave the egg, but moults inside, and finally emerges as an eight-legged nymph. It lives in the dust in the cracks of the native huts and comes out at night to feed on the sleeping natives.

As the possibilities for destruction are not so great as with many Ixodinæ the necessity for thousands of eggs is not imperative for the continuation of the species as with the Ixodinæ. With some of the Ixodinæ the females lay from 5000 to 20,000 eggs during several days or weeks and then die. The eggs are preferably deposited near grass. The egg stage lasts from two to six months, when the six-legged larva ("seed tick") emerges. It crawls up a blade of grass and gets on a passing animal. After feeding, or at times without taking nourishment, the larva drops to the ground, and changes to the pupal stage which has four pairs of legs. The pupa crawls up a blade of grass and gets on a passing animal (the second host). Feeding, it falls to the ground where it remains eight or ten weeks. It moults and develops into an adult tick. These males and females gain access to a third animal host—the males fecundate the females, after which the female gorges herself with blood; afterward dropping off the animal and laying eggs. With some ticks fewer hosts suffice.

Cleland has noted reports of serious symptoms, chiefly cardiac and visual, from the bite of ticks in Australia (*Ixodes holocyclus*). This is exceptional, however, as the symptoms following the bites of such ticks are only those of skin irritation.

Classification of Ixodidæ

Subfamily Argasinæ.—Head concealed by body when viewed dorsally. No scutum. Stigmal plates between third and fourth legs. Adults have no suckers (pulvillus) beneath claws. Slight sexual dimorphism. Anus near middle of venter. Skin rough.

Genus Argas.—Body narrow in front. Margins thin and acute. No eyes. The *A. persicus* (Miana bug) of Persia has been supposed to be concerned in the transmission of a serious disease. Rostrum some distance from anterior margin. It is also called the fowl tick and transmits fowl spirillosis.

Genus Ornithodoros.—Margins of body rounded. Skin has many irregular tubercles. Rostrum even with anterior margin so that ends of palpi slightly project. It is the intermediate host of *Spirochæta duttoni*. (South African tick fever.)

O. moubata is very common in Africa living in cracks in mud floors and bites severely the sleeping natives. The larva makes its first moult inside the egg so that it shows four pairs of legs when it emerges. Christy thinks it may transmit *Filaria perstans*.

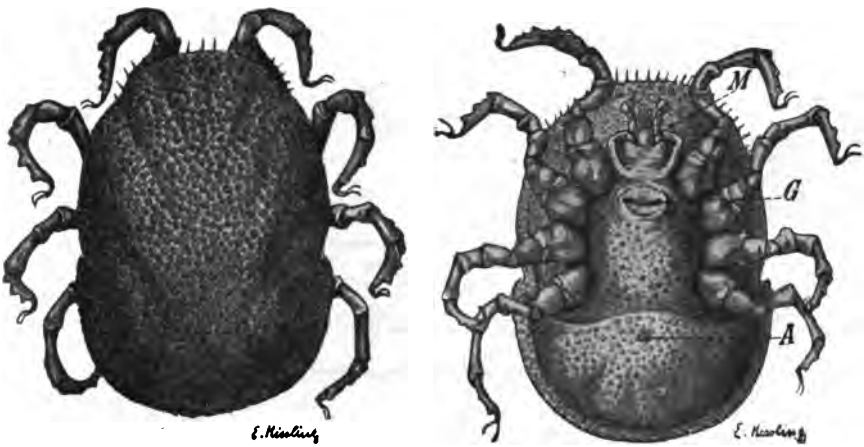


FIG. 101.—*Ornithodoros moubata*. (*Murray from Doflein.*)

Tick Fever.—With tick fever the epidemiology rests upon the life history of the tick *O. moubata*. This tick infests the rest houses along the route of travel, hiding in the crevices of floors and walls during the day and coming out at night to bite the sleeping inmates. The feeding occupies a long time, more than an hour. Both sexes bite man.

The female lays about 100 eggs, from which a nymph emerges in about twenty days. The larval stage takes place in the egg. Shortly after emerging the nymphs suck blood. An important fact is that the female transmits the spirochæte to its ova, so that the nymphs may transmit the disease.

Natives seem to suffer severely from tick fever in childhood but in adult life possess a sufficient degree of immunity so that the disease shows itself in a very mild form in those harboring spirochætes. Ticks can be infected by these carriers. In some of the rest houses 50% of the ticks may be infected. While the tick does not tend to leave its habitation it may be transported in the bundles of native porters.

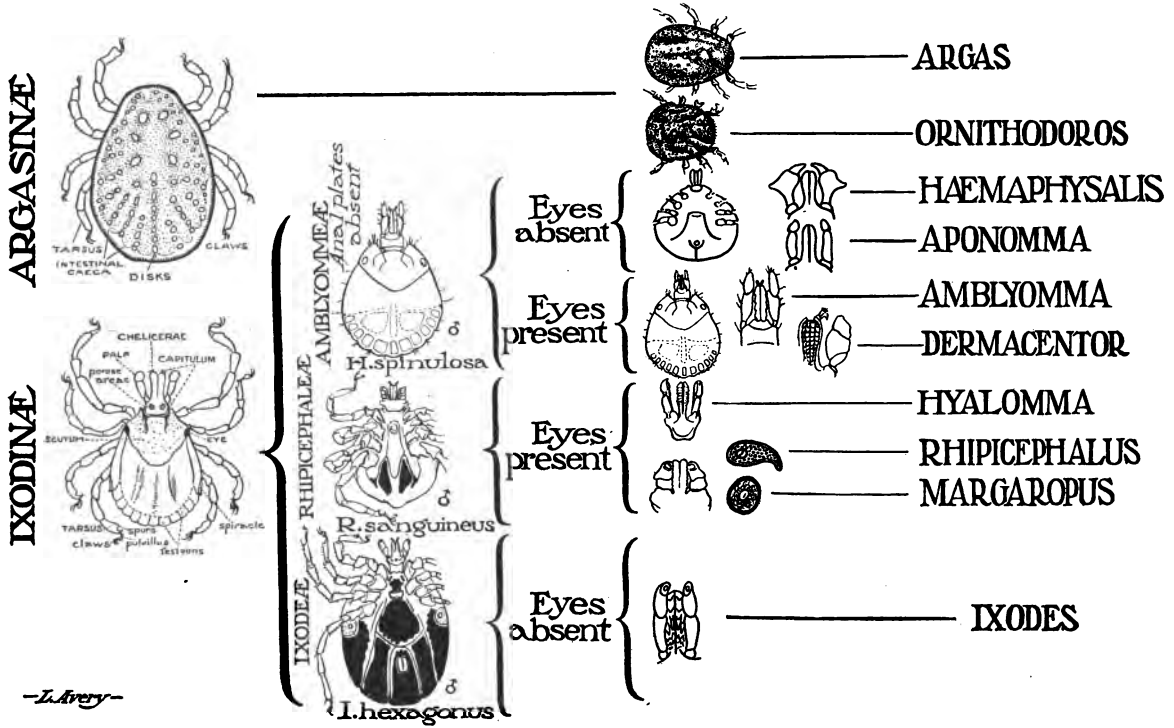


FIG. 102.—Diagrammatic key to ticks, modified from Lahille. Includes Ixodeæ (Males clothed on all their ventra surface with anal plates in uneven numbers. Rostrum elongate. No eyes). Rhipicephaleæ. (Males having anal plates in pairs. Rostrum may be long or short. Eyes present.) Amblyommeæ. (Males without anal plates.)

The transmitting agent of the north African relapsing fever and probably of the Indian type is the louse. The body louse deposits about 75 eggs in the clothes of the host, which hatch out in about four days and become adults in about two weeks. The head louse deposits its eggs or nits on the hair of the host's head. (See Pediculus.)

O. savignyi has two pairs of eyes near base of mouth parts.

Subfamily Ixodinæ.—Mouth parts project in front of body when viewed dorsally. Scutum present. Stigmal plates posterior to fourth pair of legs. Adults have suckers beneath claws. Skin finely striated.

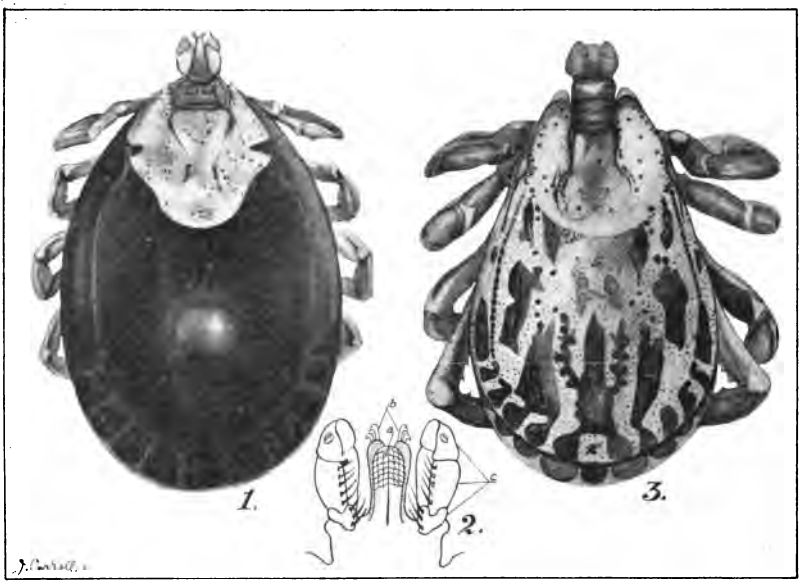


FIG. 103.—1. Female D. andersoni. 2. Head showing (*a*) hypostome, (*b*) cheliceræ, (*c*) palps. 3. Male.

Anus behind middle of venter.

Sexual dimorphism marked. Male has well-developed scutum; female has porose areas.

Section Ixodæ.—Transverse recurved preanal groove in female. Male has ventral surface covered with chitinous plates. No eyes. Genus *Ixodes*.

Ixodes has long rostrum with slender palpi—palpi narrow at base, leaving gap between them and hypostome.

Section Rhipicephalus.—No preanal, but postanal groove in female. Ventral surface of male without adanal plates in *Dermacentor, Hæmaphysalis, Aponomma*

and *Amblyomma*, but with one or two pairs in *Hyalomma*, *Rhipicephalus* and *Margaropus*.

In the genera *Hyalomma*, *Aponomma* and *Amblyomma* the palpi are long and slender and of about uniform width of segments.

In *Hyalomma* the segments of palpi are of about equal length. In *Aponomma* and *Amblyomma* the second palpal segment is much longer than the others. *Amblyomma* differs from *Aponomma* in being very ornate and in having eyes.

In the genera *Hæmaphysalis*, *Dermacentor*, *Rhipicephalus*, and *Margaropus* the palpi are short.

Hæmaphysalis has very broad rostrum, triangular palpi, and no eyes. *Dermacentor* has a square rostrum with short thick palpi, the second and third joints being as broad as long. *Dermacentor andersoni* transmits spotted fever of the Rocky Mountains—not *D. reticulatus*. Banks states that the proper name is *D. venustus*. Rocky Mountain fever is discussed in Chapter XXXVI. In British Columbia the bite of this tick is said to cause a paralysis. The most important characteristic of the genus *Dermacentor* is the large size of the coxæ of the fourth pair of legs.

Rhipicephalus has palpi without transverse ridges and comma-shaped stigmal plates. The stigmal plates of *Margaropus* are nearly circular and the palpi have acute transverse ridges externally. *Margaropus annulatus* transmits Texas fever of cattle. This tick is also called *Boophilus bovis* or *B. annulatus*. Some authors term it *Rhipicephalus annulatus*. Larvæ developing from eggs of female ticks which have fed on cattle infected with Texas fever transmit the disease which is due to a protozoon *Babesia bigemina*.

PENSTASTOMIDA (TONGUE WORMS)

These are vermiform acarines more or less distinctly annulated. They have retractile hooks at either side of the elliptical mouth.

If the hooks are to be considered not as degenerated legs but antennæ and palpi, then there is no vestige of legs in the adult. The sexes are separate.

Linguatula rhinaria.—This has been observed in man both in larval and adult stages.

The male is white and about ¾ inch long while the female is about 4 inches long, tadpole shape, yellowish in color, and has about 90 segments, lives in the nasal cavity and frontal sinus of dogs, rarely in horses and sheep, and very rarely in man.

The female lays embryo-containing eggs which, gaining freedom through the nasal mucus, are swallowed by various animals. A larva develops which bores its way through the gut and encysts in the liver or mesenteric glands. After several moultings, they work their way again to the intestines and so get out of the body of their host; or they may wander to lungs and trachea and either escape or take up their position in the nostrils to become adults and produce eggs. Consequently, one animal may act as intermediate and definitive host or these cycles may take place in distinct animal hosts.

The larval form (⅕ inch) is far more common in man than the adult. Symptoms are referred to liver in both larval and adult stage, and epistaxis and nasal symptoms for adult stage only.

Porocephalus constrictus.—The adult form *P. moniliformis* lives in the lungs of snakes and the eggs are probably ingested by drinking water. These eggs develop into a curled-up, ringed larva, about ½ inch long with 23 rings, which is encysted especially in the liver or lungs. These escape and are swallowed by the snakes, their definitive hosts.

While in the liver or lungs of man the patient may have signs of bronchitis, hepatitis or peritonitis. Cases usually only discovered at postmortem. Parasites, however, might possibly be found in sputum or fæces.

CHAPTER XX

THE INSECTS

CLASSIFICATION OF THE CLASS INSECTA

Order	Family	Subfamily	Genus	Species
Siphunculata	Pediculidæ		Pediculus	P. capitis
				P. vestimenti
			Phthirius	P. pubis
Rhynchota (Hemiptera)	Acanthiidæ		Acanthia	A. lectularia
	Reduviidæ		Conorhinus	C. megistus
				C. sanguisuga
Siphonaptera	Pulicidæ	Pulicinæ	Pulex	P. irritans
			Xenopsylla	X. cheopis
			Ceratophyllus	C. fasciatus
			Ctenocephalus	C. serraticeps
			Ctenopsylla	C. musculi
		Sarcopsyllinæ	Sarcopsylla	S. penetrans
Diptera	Simulidæ (buffalo gnats)		Simulium	S. reptans
	Psychodidæ (moth midges)		Phlebotomus	P. papatasii
	Chironomidæ (midges)		Ceratopogon	C. pulicaris
	Culicidæ	Culicinæ	Stegomyia	S. calopus
			Culex	C. fatigans
		Anophelinæ	Anopheles	A. maculipennis
	Tabanidæ (horseflies)		Tabanus	T. bovinus
			Hæmatopota	H. pluvialis
			Pangonia	P. beckeri
			Chrysops	C. silacea
	Muscidæ		Glossina	G. palpalis
				G. morsitans
			Stomoxys	S. calcitrans
			Musca	M. domestica
			Auchmeromyia	A. luteola
			Calliphora	C. vomitoria
			Lucilia	L. cæsar
			Chrysomyia	C. macellaria (screw-worm)
	Sarcophagidæ		Sarcophaga	S. carnaria
			Ochromyia	O. anthropophaga
	Œstridæ		Dermatobia	D. cyaniventris
			Hypoderma	H. diana

INSECTA

The class Insecta has one pair of antennæ, three pairs of mouth parts (the fused labium being considered as one pair), and three pairs of legs. They have three divisions of the body—head, thorax, and abdomen.

The head carries the antennæ and mouth parts; the thorax, which is divided into the pro meso and meta thorax, carries upon the ventral surface of each thoracic segment a pair of legs and on the dorsal surfaces of the two posterior segments a pair of wings. The abdomen does not support appendages. The air is supplied by means of tracheæ—branching breathing tubes which have external openings or stigmata. The tracheæ are stiffened by spiral chitinous bands. The Malpighian tubules are excretory organs of the alimentary system and excrete nitrogenous waste material. Insects have two pairs of wings, the second pair of which is frequently rudimentary and shows simply as knob-like projections. These are termed halteres or balancers. In some insects both pairs of wings are rudimentary, as in Siphonaptera.

Where insects show metamorphosis we have voracious worm-like larvæ coming out of eggs; these larvæ are succeeded by a quiescent nonfeeding encased pupa which finally develops into an imago or fully developed insect. An insect which does not present this developmental cycle shows incomplete metamorphosis. Of the class Insecta only the Siphunculata, Rhynchota, Siphonaptera, and Diptera are of special importance.

SIPHUNCULATA (ANOPLURA)

These are small dorso-ventrally flattened wingless insects not showing metamorphosis.

The Pediculidæ

In this family there are no wings and there is no metamorphosis. They have simple eyes and 5 joints to the antennæ. The legs are well developed and terminate in powerful claws. The young resemble the adults. The acorn-shaped eggs (nits) are deposited on hairs or clothing of the host.

Pediculus capitis.—The female is about 1/12 inch (2 mm.) long; the male smaller. They vary in color according to the color of the hair of the host. The eggs are deposited on the hairs of the head in number of 60 which hatch out in about six days. The thorax is as broad as the abdomen. The male louse is rounded off posteriorly and shows a dorsal aperture for a pointed penis, while the female is recognized by a deep notch at the apex of the last abdominal segment. There seems to be a marked preference exhibited by lice for their own peculiar racial host. It has been suggested that this might account for certain peculiarities in infection where different races were living together and under similar conditions as to food and environment, and yet only one race contracts the disease (beriberi). The head louse has been found to harbor leprosy bacilli when living on a leper.

Pediculus vestimenti.—This louse lives about the neck and trunk underclothing and deposits its eggs in the clothing. They feed on the host about twice daily, but are rarely found on the skin. The eggs usually number about 75 and hatch out in seven to ten days when on clothing worn next the body and become mature in about two weeks. The mature female deposits these eggs to the number of four or five daily so that in her average life of four or five weeks she lays the number noted above. Feeding takes place about twice daily and deprivation of food will kill the adult in five days. The newly hatched louse will die in two days if unfed. Lice eggs remain viable for more than a month in clothing which has previously been worn.

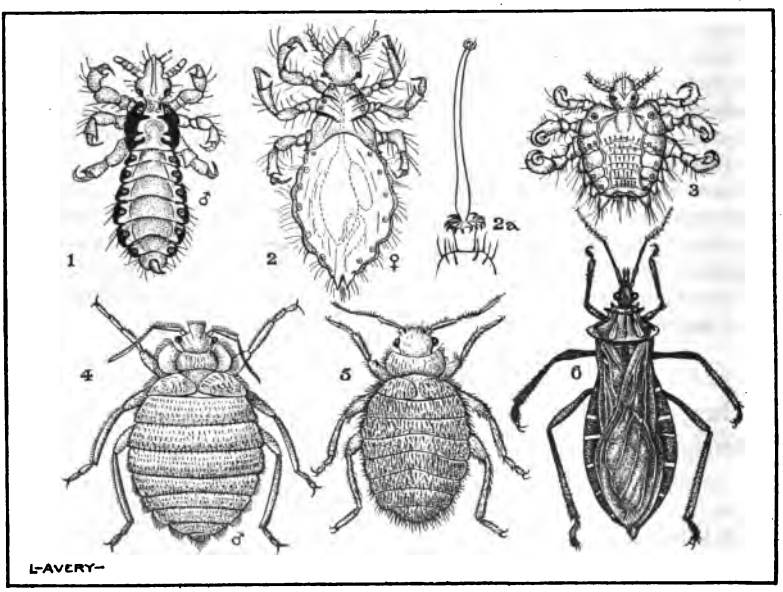


FIG. 104.—Siphunculata and Rhynchota. 1. *Pediculus capitis.* 2. *Pediculus vestimenti.* 2a. Protruded rostrum of *Pediculus.* 3. *Phthirius pubis.* 4. *Acanthia lectularia.* 5. A. rotundata. 6. *Conorhinus megistus.*

As these eggs are generally deposited in seams such clothing should be steam treated before being again worn to destroy the eggs. The females do not begin to oviposit for three or four days after reaching maturity and before doing so must feed. If they experience a temperature below 65°F. they will not oviposit. The female lives about six weeks and during that time may lay as many as 300 eggs. Unlike the fleas there is no grub stage.

It is almost twice the size of the *P. capitis* and the abdominal segment is broader than the thorax. The abdomen is less markedly festooned than that of *P. capitis;* is less hairy and contains 8 segments as against 7 for *P. capitis.*

It has recently been shown to transmit typhus fever and more recently Nicolle has demonstrated it as a carrier of relapsing fever, the spirochætes being introduced by the material from the crushed louse being rubbed into the wound by the scratching of the victim (just as with the flea in plague) and not by the bite itself. It is also supposed to transmit the trench fever infection. The dog louse as well as the dog flea serves as an intermediary host for *Dipylidium*.

Phthirius pubis.—This louse is popularly known as the crab louse. The female is little more than $\frac{1}{25}$ inch in length, and the male a trifle less. They are almost square. The second and third pair of legs are supplied with formidable hooks. They have a preference for the white race and live about the pubic region. The female lays about a dozen eggs, which hatch out in about a week

FIG. 105.—Female *Pediculus corporis*.—(*Schamberg, After Kuechenmeister.*)

RHYNCHOTA

The Rhynchota are insects possessing a sucking beak in which the lower lip forms a long thin tube or rostrum which can be bent under the head or thorax. Inside this tube are biting parts —mandibles and maxillæ. The metamorphosis in this order is not marked.

They have no palpi. The lower lip or labium or beak has its edges curved to form the tube and it is only covered by the labrum at its base. With the Diptera the labrum goes into the formation of the sucking tube. The mandibles and maxillæ are bristle-like structures serrated at the tip. The mandibles are grooved internally and form when apposed a tube for blood.

The Acanthiidæ

These have a flattened body, a three-jointed rostrum, and four-jointed antennæ. Their wings are atrophied.

Acanthia lectularia (**Cimex lectularius**).—This is the cosmopolitan bedbug. It measures about $\frac{1}{5}$ by $\frac{1}{8}$ inch (5 by 3 mm.). It is of a brownish-red color. The most conspicuous feature of the bedbug is the long proboscis continuous with the dorsal integument of the head and tucked under the ventral surface. There are two prominent eyes and two four-jointed antennæ. There are eight abdominal segments. The bedbug lives in cracks and crevices, especially about beds. It is said they can migrate from house to house. At any rate, they are frequently transferred with wash clothes. They have a penetrating odor when crushed. The female deposits about 50 eggs at a time in cracks and in ten days they hatch out into larvæ which pass insensibly into adults by a series of five moultings; this depositing of eggs occurs about four times a year.

The bedbug is very probably the intermediate host in kala-azar and it has been incriminated in connection with typhus fever and relapsing fever. It can also transmit plague.

A. rotundata.—In India the *A. rotundata* is the one encountered. It is of a dark mahogany color, has a smaller head, narrower abdomen, thick rounded prothoracic borders and is more densely covered with hairs than *A. lectularia*. The prothorax of *A. lectularia* is flattened at the side.

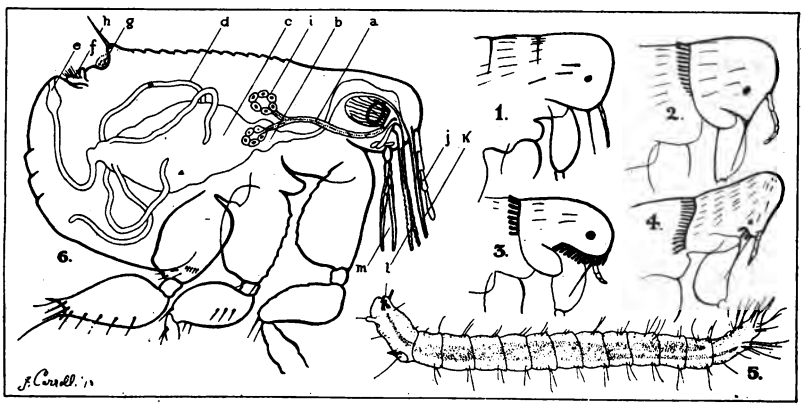


FIG. 106.—1. *Xenopsylla cheopis*. 2. *Ceratophyllus fasciatus*. 3. *Ctenocephalus felis*. 4. *Ctenopsylla musculi*. 5. Flea larva. 6. Internal anatomy of flea: a, salivary duct; b, proventriculus; c, stomach; d, malpighian tubules; e, rectum; f, claspers; g, pygidium; h, antipygidial bristles; i, salivary ducts; j, maxillary palps; k, epipharynx; l, mandibles; m, labial palps.

Reduviidæ

These bugs have a long narrow head and a distinct neck. The antennæ are long and slender. The antennæ in the genus *Conorhinus* are inserted about midway between the eyes and point of the head.

Conorhinus sanguisuga.—This is known as the Texas or Mexican bedbug, and was formerly the foe of the common bedbug, but having gotten a taste for human blood through the *Cimex* or *Acanthia*, it now prefers man. It is extending toward the North. It has wings. The bites are much more severe than those of the common bedbug. It is of a dark brown color, nearly an inch in length, with a long, flat, narrow head and a short thick rostrum. They can run as well as fly. They bite at night.

Conorhinus megistus.—This is called "Barbeiro" in Brazil on account of its preference for biting the face. The *Schizotrypanum cruzi* undergoes a developmental cycle in this bug which transmits the disease.

SIPHONAPTERA

These are laterally flattened, markedly chitinized, wingless insects which undergo a complete metamorphosis.

Pulicidæ

This family is divided into two subfamilies—the Pulicinæ and the Sarcopsyllinæ. In the former the female remains practically unchanged with freedom of movement after fecundation, while in the latter the abdomen becomes enormously distended with eggs, and the female remains fixed in the burrow which she has made under the skin.

Pulicinæ.—Formerly, with the exception of infection with *Dipylidium caninum*, the fleas were only under suspicion as carriers of disease; ideas having been entertained as to their being possible transmitters of relapsing fever, typhus fever and kala-azar. *Trypanosoma lewisi* is transmitted by fleas, either *Pulex irritans* or *C. canis*. The trypanosome undergoes development in the flea and the infecting material is in the fæces of the flea and transmission occurs by the licking on the part of the rat of fæces from an infected flea. The infection has no connection with the puncture wound of the flea as is the case with plague. As a result of the convincing experiments of the Indian Plague Commission, their rôle in the transmission of plague has been absolutely established. It is by the bite of the *Xenopsylla cheopis* that plague is chiefly transmitted from rat to rat, and in bubonic and septicæmic plague it is apparently the intermediary in human infection. Any species of flea which lives on the rat is capable of transmitting plague as would also be the case if *Pulex irritans* fed on the blood of a human case of septicæmic plague.

The average capacity of a flea's stomach is about 0.5 cu. mm. so that with a rat dying with septicæmic plague and with possibly 100,000,000 bacilli to 1 c.c. of blood the flea would take in about 5000 bacilli. Furthermore these multiply in the alimentary canal so that the digested blood teems with bacilli when reaching the anus of the flea. The plague bacilli are passed out with the fæces and these being rubbed into the puncture of the flea bite bring about infection. Regurgitation as result of obstruction by masses of plague bacilli in the œsophagus causes injection of plague bacilli into rat or man in the act of biting. This is more important than the fæces inoculation method. The puncturing apparatus of the flea consists of a pointed epipharynx and two distally serrated mandibles. These chitinous biting parts are contained in the labium which divides distally into two labial palps. The maxillæ are conspicuous triangular structures and, projecting farthest anteriorly, are the conspicuous four-jointed maxillary palps, often mistaken for antennæ. By the apposition of the internally grooved mandibles to the epipharynx a tube is formed through which the blood is sucked up. The antennæ are inconspicuous and are in close apposition to the sides of the head, behind the eyes, and can only be well made out with a lens. Fleas have three pairs of legs, and the male can be distinguished from the female by its smaller size and the conspicuous coiled-up penis within the abdomen. The female has a conspicuous gourd-like spermatheca

which varies in shape in different species. A very prominent structure is a pitted plate in the ninth abdominal segment (pygidium). Of importance in classification are prominent bristles originating from the seventh abdominal segments and projecting over the pygidium. These bristles vary in number and are known as antipygidial bristles.

The body of the flea is flattened laterally. They may or may not have eyes, and certain conspicuous structures called combs are of importance in classification. In the metamorphosis of the flea the eggs are hatched out in dust of crevices, etc., into bristled larvæ in about one week. The larva forms a cocoon and develops into a nymph which has three pairs of legs. The nymphs emerge from the cocoon as adult fleas in about three weeks after the larva forms it.

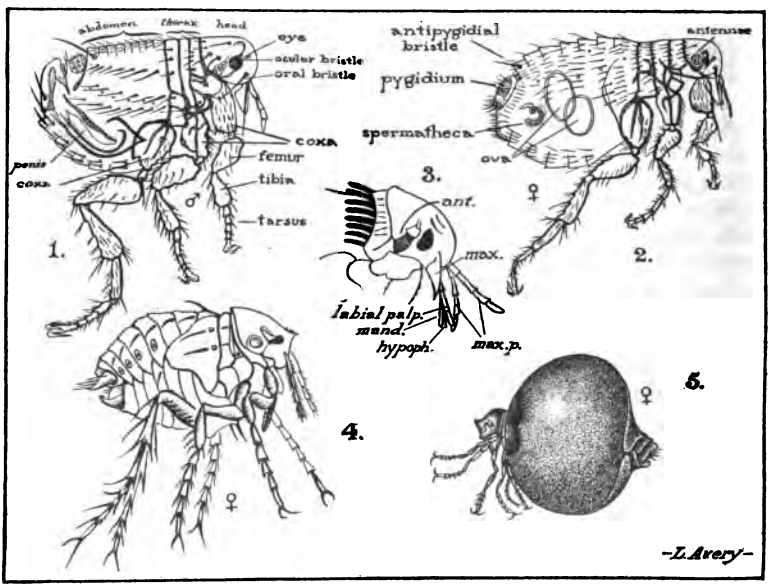


FIG. 107.—1 and 2, male and female *Xenopsylla cheopis*. 3, Head of *Ceratophyllus*. 4 and 5, male and egg distended female of *Sarcopsylla penetrans*.

KEY TO THE FLEAS

A. With combs.

1. Eyes present.

(*a*) Combs along inferior border of head and on prothorax. ***Ctenocephalus serraticeps.***

(*b*) Combs only on prothorax. ***Ceratophyllus fasciatus*** (with only one antipygidial bristle on each side).

Hoplopsyllus anomalus (with many antipygidial bristles).

2. Eyes absent.
   (*a*) Collar of combs on prothorax and four short ones along inferior border of head. *Ctenopsylla musculi.*

B. Without combs.
   (*a*) Ocular bristle arises near upper anterior margin of eye. A line between this and the oral bristle approximately vertical. Two bristles posterior to antennæ. *Xenopsylla cheopis. Loemopsylla cheopis.* Formerly *Pulex cheopis.*
   (*b*) Ocular bristle arises near lower anterior margin of eye. A line between this and the oral bristle approximately horizontal. One bristle posterior to antennæ. *Pulex irritans.*

The common human flea of Europe is the *Pulex irritans;* that of the United States the *Ctenocephalus serraticeps* or dog flea. The flea that is prominently implicated with plague is the *Xenopsylla cheopis*, on account of its being the common rat flea of India, where it has been much studied. It resembles *P. irritans*, but is more yellow than brown in color. It also has a greater number of bristles on the head. The ocular bristle runs above and in front of the eye; that of *P. irritans* below. It is principally the flea of *Mus decumanus* (*M. norvegicus*), the sewer rat; but the house rat, *M. rattus*, becomes infected from coming in contact with the sewer rat in the basement. In the U. S. the ground squirrel, *Citellus beechyi* acts as a reservoir of plague and has as its flea *Hoplosyllus anomalus.*

Ceratophyllus fasciatus is the common rat flea of Europe and the U. S. In the tropics *X. cheopis* is the common rat flea (98% in India). *Ctenocephalus serraticeps*, *Ctenopsylla musculi* and *Pulex irritans* have also been frequently found on both *Mus norvegicus* and *M. rattus.* To distinguish *M. norvegicus* from *M. rattus* we have in the former (1) ears which barely reach the eyes when laid forward and (2) tail rather shorter than length of head and body together (only 89% of length of head and body together). With *M. rattus* the tail is longer than the head and body together (25% longer) and the extended ear covers or reaches beyond the middle of the eye. *M. rattus* has a sharper nose, longer and more delicate tail and thinner ears than *M. norvegicus* (formerly *M. decumanus*).

M. alexandrinus is a variety of *M. rattus.* Rats and mice belong to the family Muridæ and the common mouse is *M. musculus.* They belong to the order of Rodentia of the class Mammalia.

Sarcopsyllinæ

Belonging to the subfamily Sarcopsyllinæ, the *Sarcopsylla penetrans* (*Dermatophilus penetrans*) is of great importance in tropical countries. It is known as the chigoe, nigua, or jigger. The male and virgin female are unimportant as they do not penetrate the skin but act as ordinary fleas. The female, which when unimpregnated is only about $\frac{1}{24}$ inch long, when impregnated bores its way into the skin of man, especially about the toes, soles of the feet or finger-nails, and in the chosen site develops enormously, becoming as large as a small pea. This enlargement takes place in the second and third abdominal segments and is packed with eggs measuring about 400 microns long and numbering about 100. A small black spot in the center of a tense rather pale area is characteristic. The metamorphosis is

similar to that of the flea. *Sarcopsylla* can be differentiated from the flea by the proportionately larger head to the body, and especially by the fact that the head is the shape of the head of a fish, distinctly pointed. With the fleas the lower border of the head comes out in a straight line to join the curve of the upper part. In the *Sarcopsylla* lower and upper border of head are both curved.

Diptera

The insects of the order Diptera are of great importance medically in a variety of ways, either by the direct irritation of their bites, by their transmitting disease directly, as does the common house fly typhoid fever, or by acting as intermediate or definitive hosts for various parasites. They are characterized by mouth parts formed for puncturing, sucking, or licking. They present a complete metamorphosis, larva, pupa, and imago. As a rule, the Diptera have a distinct pair of wings the second pair being rudimentary.

The order Diptera is usually divided into the following suborders: 1. *Orthorrhapha:* Diptera with larvæ having a differentiated head. The imago breaks through the larval or pupal case by a T-shaped break and has no frontal lunule (an oval space just above the root of the antennæ). The Orthorrhapha are divided into: *a.* Nemocera (with long, many jointed antennæ) and *b.* Brachycera (with short antennæ).

The Nemocera are generally midge-like insects and have, as a rule, long slender palps (mosquito). The Brachycera, however, are seldom midge-like. The antennæ are composed of only two or three simple joints with or without style or arista. The palps are almost always short and never more than two-jointed. 2. *Cyclorrhapha:* larvæ without differentiated head. The imago escapes through an anterior opening and has a lunule and ptilinum (an inflatable projecting organ just above the root of the antennæ). If the halteres are covered by a scale (squama) we have calyptrate Cyclorrhapha; if not, acalyptrate. These squamæ are large enough in the calpytrate species to even conceal the halteres when the fly is looked at from above. 3. *Pupipara:* the larvæ are extruded from the mother and almost immediately begin the pupal life. Leathery flies with poorly developed wings (Hippoboscidæ).

The males of flies where the two compound eyes come together above the antennæ are referred to as holoptic, if more or less widely separated as dichoptic. Ocelli are three single eyes usually, when present, situated in the triangular space between the compound eyes in the frons (the space separating the compound eyes).

The anterior portion of the head which lies below the origin of the antennæ is the face and on each side of the face we have the cheeks which should be studied as to presence of abundance of hairs. The antennæ which separate the frons from the face are of great importance in classification. In the Muscidæ the appearance of a feathery structure, projecting from the terminal segment of the antennæ, and called

the *arista*, is important. This may be bare or feathered and the feathering may be only on one side or of one part.

In studying the biting flies it is very important to recognize the anterior, small, or mid-cross vein. This short transverse rib or vein is the key to wing venation. Beneath it is the discal cell and it bounds the first posterior cell internally or basally. The fourth longitudinal vein, which touches the bottom of the mid-cross vein, is of particular importance as it gives different shapes to the first posterior cell as it runs along the lower border of this cell. The closed-in discal cell is below the fourth longitudinal vein. The character of the antennæ should also be noted carefully. The study of the bristles about head, thorax, and abdomen (chætotaxy) is more difficult. Anyone taking up the study of flies should carefully note the wings, etc., of *Musca domestica*. By putting a few house flies on moist horse manure in a gauze-covered bottle the entire metamorphosis may be observed.

Tabanidæ

This is the family of horseflies, gadflies, breeze flies or green-headed flies. It is the most numerous family of the Diptera—there being more than 1000 species. The females are blood suckers; the males live on flowers and plant juices. The eyes are usually very brilliant in color, and in the male make up the greater part of the head.

They belong to the suborder Orthorrhapha and in the group of short antennæ flies (Brachycera). Five posterior cells are always present.

The antennæ consist of three segments. No arista. The epipharynx is tube like, the hypopharynx has a groove and both are awl-shaped. The pair of maxillæ are serrated and the mandibles lancet like. They have rather coarse maxillary palps. The labellæ are prominent at the extremity of the fleshy labium. They are thick set flies and rarely show color. The body of the larva has eleven segments with a small but distinct head. The eggs are deposited in masses on the leaves or stems of plants about marshy places. The larva is carnivorous.

Tabanus autumnalis.—Is about ¾ inch long; it is dark in color, and has four longitudinal bands on the thorax. The last joint of the antennæ has a crescentic notch. The wings do not overlap.

Hæmatopota pluvialis.—In the *Hæmatopota* there is no crescentic antennal notch, and the wings overlap. The abdomen is narrower than in *Tabanus*. The brimp, one of the *Hæmatopota*, bites man severely.

Pangonia beckeri.—The genus *Pangonia* is characterized by a very long, slender, and more or less horizontal proboscis.

Chrysops dispar.—*Chrysops* has three ocelli, in this respect differing from the genera *Tabanus* and *Hæmatopota*. The wings are widely separated and spotted. The antennæ of *Chrysops* are especially long and slender. *Chrysops* and *Hæmatopota* produce the greatest amount of pain from their bites. The *Tabanidæ* except *Chrysops* are not implicated as intermediate hosts in the transmission of disease. By their bites, however, they may transmit disease directly, as with anthrax. Two species of *Chrysops*, *C. dimidiata* and *C. silacea*, have been found to transmit *Filaria loa*.

Muscidæ

The Muscidæ, Sarcophagidæ, and Œstridæ are calyptrate Cyclorrhapha.

Musca domestica.—The common housefly, *Musca domestica*, is the best example of this family.

The arista is feathered both dorsally and ventrally with straight hairs. The fourth longitudinal vein bends down in a rather sharp angle as compared with *Stomoxys*, which gives the first posterior cell of the latter rather a fusiform appearance. The eyes are close together in the male, far apart in the female. The female lays about 125 eggs in a heap preferably in fermenting horse manure. The larva comes out in about thirty-six hours. Very characteristic are the stigmata decorating the blunt posterior ends. (See illustration.)

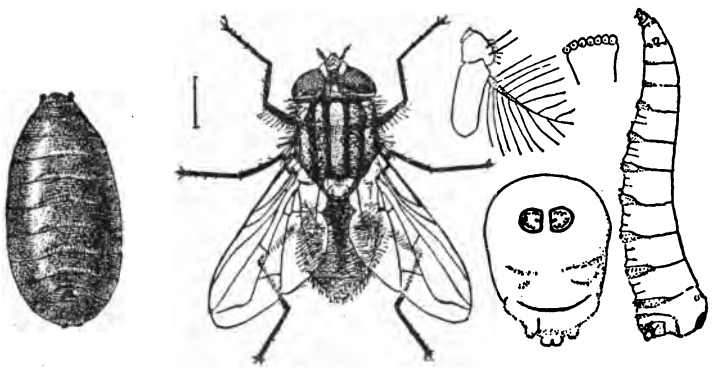


FIG. 108.—Common housefly (*Musca domestica*): Puparium at left; adult next, larva and enlarged parts at right. All enlarged. From circular 71 (by L. O. Howard), Bureau of Entomology, U. S. Department of Agriculture.

The larval stage lasts seven to ten days and then the barrel-shaped pupal stage is entered upon. This lasts about three days when the adult fly emerges. This is termed a "coarctate" pupa. The larva shrinks and is surrounded by its old skin which is termed a puparium. This fly is incapable of biting, the piercing organs being fused with the labium, but may transmit disease directly, carrying infectious material from the source, as in fæces, to the food about to be ingested. Their rôle in typhoid fever is one of immense importance. By reason of its hairy sticky legs, habits of frequent defecation and constant regurgitation the housefly is an important agent in the spread of cholera, dysentery, infantile diarrhœas and tropical ophthalmias as well as typhoid.

In the Muscidæ the antennæ hang down in front of the head in three segments and have an arista plumose to the tip. The first posterior cell is narrowed. There are no bristles on abdomen except at tip.

(I) *Stomoxys*, *Hæmatobia* and *Glossina* have a more or less elongated proboscis adapted for biting. *Stomoxys* has delicate palpi, shorter than

the proboscis, and arista feathered only on the dorsal side with straight hairs. *Hæmatobia* has club-like palpi about as long as proboscis and arista with hairs dorsally and ventrally. *Glossina* has thick set but not clubbed palpi and an arista feathered on the dorsal side with branching hairs.

(II) *Musca, Calliphora, Chrysomyia, Lucilia,* and *Cordylobia* do not have a proboscis adapted for biting.

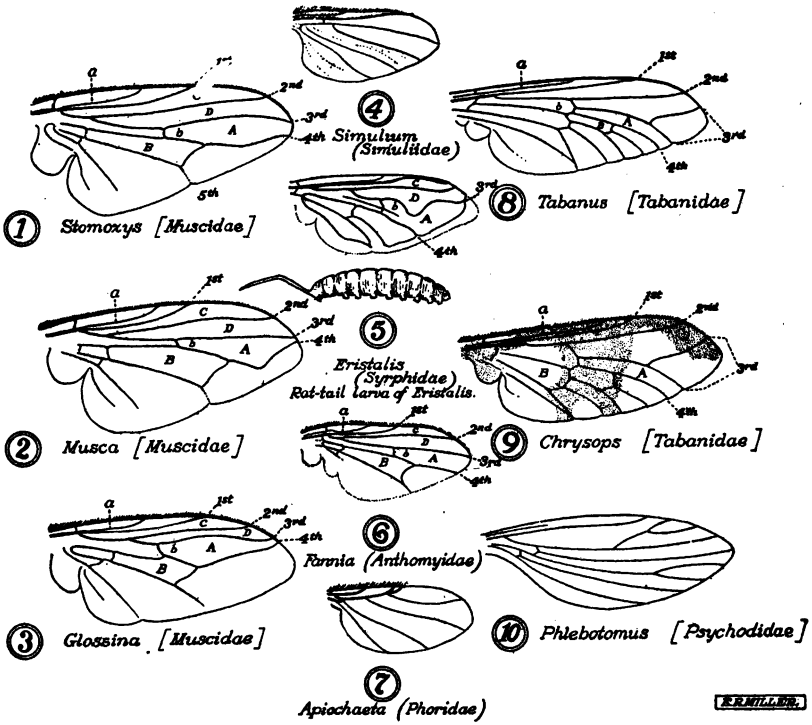


FIG. 109.—Wing venation of Diptera. *A*, First posterior cell; *B*, discal cell; *b*, mid cross-vein; *a*, auxiliary vein; *C*, marginal cell; *D*, submarginal cell.

Stomoxys calcitrans.—These greatly resemble the common housefly in size and shape. They can be easily distinguished by the black, piercing proboscis extending beyond the head. There are longitudinal stripes on the thorax and spots on the abdomen. The proboscis on examination will be seen to be bent at an angle near its base. The palps are short and slender. The wings diverge widely.

The female lays about 60 banana-shaped eggs in horse manure. These hatch out in three days as larvæ which turn into pupæ in two or three weeks. After about

ten days the fly emerges. The genus *Stomoxys* includes vicious biters. This is the fly which comes into houses before a rain, and which has given the common housefly the reputation of biting before a rain. *Stomoxys* may be implicated in transmitting surra (*Trypanosoma evansi*).

It assumed great importance as a transmitter of poliomyelitis and possibly of pellagra a few years ago—views now discredited.

The horsefly (*Hæmatobia irritans*) rarely bites man. In these the palpi are much longer than in *Stomoxys*, being as long as proboscis. These palps are also thick and spatulate.

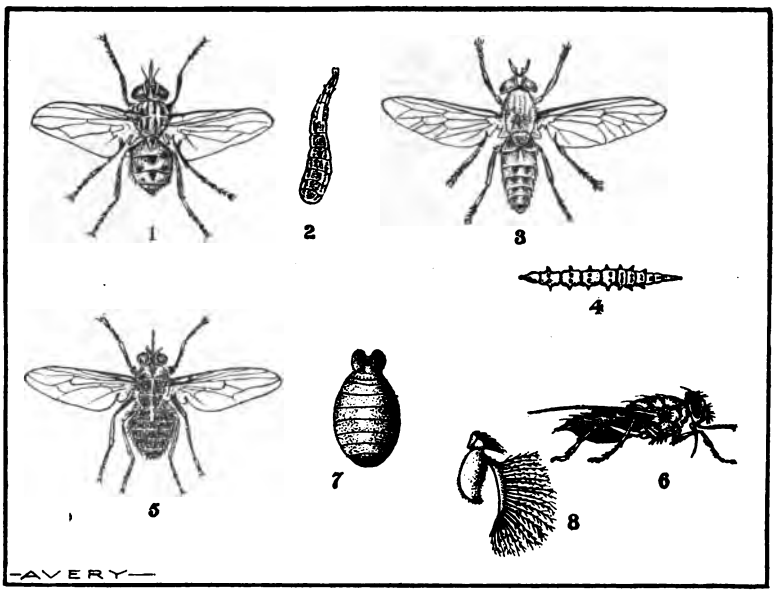


FIG. 110.—Insects in which the adult stage is important. (1) *Stomoxys calcitrans;* (2) *S. calcitrans*, larva; (3) *Tabanus bovinus;* (4) *Tabanus* larva; (5) *Glossina palpalis;* (6) *G. palpalis*, side view; (7) *G. palpalis pupa;* (8) *Glossina* palps and arista.

Glossina palpalis.—This is the tsetse fly that is responsible for the transmission of human trypanosomiasis (sleeping sickness).

The tsetse fly is a small brownish fly about ⅓ inch long. The proboscis extends vertically and has a bulb at its base. The arista is plumose only on the upper side and the individual hairs are themselves feathered. The wings are carried flat, closed over one another like the blades of a pair of scissors and project beyond the abdomen. The most characteristic feature of the tsetse fly is the way the fourth longitudinal vein bends up abruptly to meet the midcross vein and then curves

downward to run parallel with the third longitudinal vein. In *Stomoxys*, the wings separate; in *Hæmatopota* they just meet, and in *Glossina* they cross. Glossinæ bite chiefly in the daytime.

The tsetse fly is much like *Stomoxys*, but has a branching of the feathering of the arista, long palps, a bulb to the proboscis and a characteristic upbending of the fourth longitudinal vein to meet the midcross vein. The female deposits her larva near a shady place upon loose, dry, sandy soil. Moisture and sunlight are not favorable for pupal development, the sun being particularly injurious, so that pupæ, buried only an inch deep and away from shade, are killed. This fact has been utilized in prophylaxis by cutting down the trees. The trouble is that the bush growth which soon follows is favorable as shade for the pupæ.

The female gives birth to a single, yellowish brown, motile larva, which is almost as large as the mother and which, upon reaching the ground, bores its way into a coarse, sandy soil for a depth of about 2 inches and then becomes a pupa. The larval stage in the mother lasts about two weeks and the pupal stage in the ground about a month.

Male and female flies bite and transmit the disease. They bite in the daytime, usually from 9 A. M. to 4 P. M., and will bite in the sunlight.

With a view to eradication of the disease certain areas have been depopulated, but upon examining the flies caught in the district a year or more later, infected flies have been obtained. This would indicate some other reservoir than man. It is now generally conceded that the trypanosome strain in the antelope is the same as *T. rhodesiense*, both being transmitted by *G. morsitans*. Taute however believes them different as he not only injected blood containing such trypanosomes into himself, with negative result, but also allowed flies which had fed on antelopes, which were infective for laboratory animals, to feed on himself, likewise with negative result. It is a well-known fact that men in good condition are refractory to trypanosome infection so that this courageous experiment does not prove the antelope strain to be different from the human one.

One measure that has been proposed is to kill off the big game from a certain area with a view to depriving the flies of their main source of infection.

The probabilities of an animal reservoir for *T. gambiense* however is not so well settled. Many think that we may have trypanosome carriers and that such persons in the enjoyment of health may act as reservoirs of the virus. Koch suggested that crocodiles were important factors in the life of the tsetse flies and recommended the destruction of the crocodile eggs.

Glossina morsitans transmits the cattle trypanosome disease, nagana and the human infection due to *Trypanosoma rhodesiense*.

Auchmeromyia luteola.—This is an African fly, the larva of which is known as the "Congo floor maggot," and is a blood sucker. The larva is of a dirty-white color and about ⅔ inch long. It crawls out at night and feeds on the sleeping native. This is the only known instance of a blood-sucking larva.

Calliphora vomitoria and Lucilia cæsar.—These are flies with brilliant metallic-colored abdomens, commonly called blow flies in the case of *Calliphora* and bluebottle flies for *Lucilia*. They deposit their eggs on tainted meat and in wounds. Many cases of obscure abdominal trouble are probably due to the larvæ of these

flies. Intestinal myiasis is undoubtedly of greater importance than has been thought. The larvæ, with hook-like projections anteriorly and a ringed body, can easily be recognized in the fæces. They have been mistaken for flukes. They also have a tendency to be attracted by those with ozena and the larvæ may develop in the nostrils. Cheeks bare in *Lucilia*, hairy in *Calliphora*.

Chrysomyia macellaria.—This is known as the screw-worm when in the larval stage. The adult fly resembles the blue-bottle flies. It is distinguished from them, however, by the presence of black stripes on thorax. These flies are very common over nearly all North and South America. The eggs which number 250 or more, when deposited in the nostrils or in wounds, develop into the screw-worm larva, which may, by going up into the frontal sinus, cause death. These larvæ have twelve segments with rings of minute spines.

Ochromyia anthropophaga (Cordylobia anthropophaga or Tumbu Fly).—This is an African fly whose larvæ develop under the skin of man and animals. It is known as the Ver de Cayor. The larva resembles the Ver Macaque, is rather barrel-shaped and beset with small spines. It bores its way into the skin and makes a lesion like a boil which has a central opening through which the larva breathes.

Sarcophagidæ

These are known as "flesh flies." The most important characteristic is the fact that the arista is plumose up to the mid-point, beyond which it is bare. They are usually thick set and moderately large flies.

Sarcophaga carnaria.—This is a grayish fly with three stripes on thorax and black spots on each segment of the abdomen. It is viviparous. The larvæ gain access to nasal and other cavities and there develop. Cases of death have been reported. Naturally, the fly deposits its larvæ on decaying flesh. In times of war all of these flies become important by reason of "maggots" in the wound. These larvæ are the most common ones in intestinal myiases. The mouth hooklets are strongly curved and separate. Each abdominal segment has a girdle of spines. The anterior end is somewhat pointed. The hind stigmal plate is in a deep cavity.

Œstridæ

The flies of this family are usually called warble or botflies. The mouth parts are almost vestigial. They have a large head with a somewhat bloated-looking lower portion. They are often rather hairy. The larvæ which develop from the eggs are parasitic either in the alimentary canal or the subcutaneous tissues.

Dermatobia cyaniventris.—These are large, thick-set flies about $\frac{3}{5}$ inch long, with prominent head and eyes, small antennæ, and a marked narrowing at the junction of thorax and abdomen. The thorax is grayish and the abdomen a metallic blue. The larvæ are deposited under the skin in various parts of the body. When

the larvæ move they cause considerable pain. At first the larva is club-shaped, but later on it becomes oval. The former is called Ver Macaque, the latter Torcel.

Hypoderma diana.—The larval form of this fly has been reported three times for man. It forms tumors under the skin which it is thought may reach this location by proceeding in some way from the alimentary canal.

In *Hypoderma* the arista is bare while in *Dermatobia* the upper border is plumose.

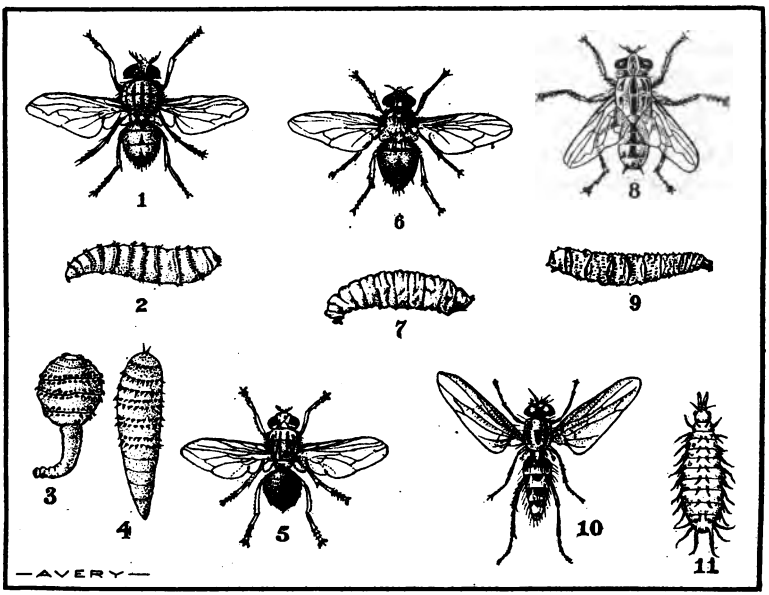


FIG. 111.—Insects in which the larval stage is important. (1) *Chrysomyia macellaria;* (2) *C.* larva; (3) *Dermatobia cyaniventris larva,* early stage (ver macaque); (4) *D. cyaniventris* larva, later stage (torcel or berne); (5) *D. cyaniventris;* (6) *Auchmeromyia luteola;* (7) *A. luteola,* larva; (8) *Sarcophaga magnifica;* (9) *S. magnifica* larva; (10) *Anthomyia pluvialis;* (11) *A. pluvialis larva.*

MYIASES

Ver Macaque.—The best known of these myiases is that due to the larva of a gadfly, *Dermatobia cyaniventris.*

The larva is at first club-shaped and in this stage is called ver macaque. Later on it becomes worm-shaped and is then called torcel in Venezuela or berne in Brazil. The natives of most of the countries where the infection is found have called the larvæ "mosquito worms" or "gusano de zancudo" and they have even incriminated large mosquitoes belonging to the genus *Psorophora* as being responsible for the infections.

Surcouf has noted that these fly larvæ have been found cemented to mosquitoes of the genus *Janihinosoma* by a glue-like substance. These mosquitoes are vicious

biters and evidently the young larvæ escape from the eggs attached to the mosquito and enter the wound made by the biting parts of the mosquito. Some have thought that *D. cyaniventris* deposits its eggs in a glue-like material on the leaves of plants and that they stick to mosquitoes flying about such plants. From the facts that these eggs apparently only become attached to this particular mosquito and further in that the eggs are attached in a constant manner with the hatching end outward it would seem that the mother fly must in some way seize the mosquito and deposit her eggs on it. As the larva grows in the subcutaneous tissues of man or other animals a tumor-like swelling develops with a central orifice, toward which the posterior extremity of the larva points and through which it takes air into its spiracles.

The swelling somewhat resembles a blind boil and may be as large as a pigeon's egg.

These gadfly boils tend to break down and discharge a sero-purulent fluid and it is supposed that the larva, when mature, escapes as a result of the disintegration of the tumor.

In Brazil they make tobacco juice applications which cause the larva to protrude and then squeeze it out. The injection of a little chloroform into the larva with a hypodermic syringe, prior to its extraction with a forceps, makes the process less painful.

The Screw-worm.—This is the larva of a blue-bottle fly, *Chrysomyia macellaria*, which differs from the common blue-bottle fly, *Lucilia*, by having three black lines on scutum.

This muscid fly lays 200 to 300 eggs in wounds or orifices having offensive discharges, as from nose, ears, etc. The larvæ burrow into the adjacent tissues and cause frightful destruction of all soft parts. The mature larvæ are a little more than ⅔ inch long and have circlets of spines around each of the 12 segments.

This infection is especially common in tropical and subtropical America and is important in animals as well as man.

Intestinal Myiases

In the tropics vague intestinal disturbances or violent abdominal cramping may be brought about by dipterous larvæ in the intestinal canal. The symptoms may be those of a dysentery and may be attended with fever and malaise.

The larvæ usually obtain access to the alimentary tract in food taken in by the mouth. Flies of the genus *Sarcophaga* are prone to deposit their larvæ on food, especially meat that is somewhat tainted. Other flies, as *Musca* or *Anthomyia*, may lay their eggs on food. Flies of the genus *Anthomyia* tend to lay their eggs on plants.

It is possible for a fly to deposit its eggs or larvæ about the anus while the man is at stool.

Great care must always be observed to assure one's self that fly larvæ, which may be present in the stool, have not originated from larvæ deposited on the stool subsequent to its passage.

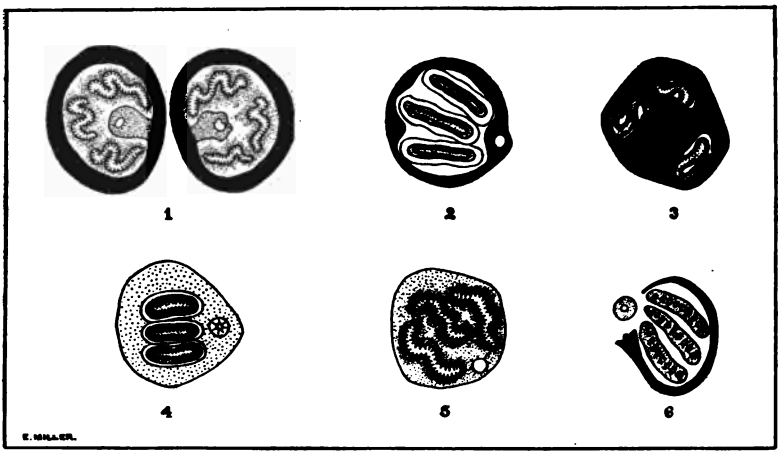


FIG. 112.—Markings of breathing slits on posterior stigmata of various dipterous larvæ. 1. *Musca domestica*, showing both stigmata; 2. *Calliphora vomitoria;* 3. *Stomoxys calcitrans.* 4. *Auchmeromyia luteola;* 5. *Cordylobia anthropophaga;* 6. *Sarcophaga magnifica.*

Aural Myiases

While the larva of *Chrysomyia macellaria*, known as the "screw-worm," is the one most frequently reported from the external auditory canal yet many such cases have been connected with the larvæ of *Sarcophaga carnaria, Calliphora vomitoria* and *Anthomyia pluvialis.* These larvæ are usually deposited in the auditory canals of those with otorrhœa.

The symptoms are intense earache, giddiness and possibly convulsions. The larvæ tend to perforate the tympanic membrane. Instillations of 10% chloroform in milk or the use of oils kill the larvæ.

DETERMINATION OF DIPTEROUS LARVÆ

There are certain points in the anatomy of dipterous larvæ which must be considered in recognition of the genus or family of the flies concerned in the various myiases. The broad extremity is the posterior

one and the tapering one the anterior. The dark hooklike processes, which may be in pairs or fused, project from the anterior or head end and above them are a pair of projecting papillæ. The second segment from the head has on either side projecting hand or fan-like structures with varying numbers of terminal divisions, 4 to 40 or more. These are the anterior spiracles.

The large terminal segment has on its posterior surface two chitinized plates with 3 slits of various architecture in each. These are the posterior stigmal plates and are the structures we pay particular attention to in identification. In the early larval stages there is only one slit; in the second stage there are two. It is only in the fully developed larval stage that we note the characteristic 3 slit stigmal plates. The presence or absence of a rounded protuberance or button at the base of each stigmal plate should be looked for. The area carrying the stigmal plates may be sunken to form a pit.

KEY TO LARVÆ OF THE MYIASES (BANKS)

1. Body with lateral and dorsal spinose processes..... Homalomyia.
   Body without such processes..... 2
2. Body ending in two fleshy processes; rather small species..... 3
   Body truncate or broadly rounded at end..... 4
3. Processes bearing the stigmal plates; body about 5 mm. long... Drosophila.
   Processes not bearing the stigmal plates; body 10 mm. or longer Piophila.
4. But one great hook; posterior stigmal plates with winding slits; no distinct lateral fusiform areas; tip of body with few if any conical processes..... Muscinæ.
   With two great hooks; slits in the stigmal plate not sinuous.... 5
5. No tubercles about anal area; no distinct processes around stigmal field..... 6
   Distinct tubercles above anal area; often processes around stigmal field; lateral fusiform areas usually distinct..... 7
6. Stigmal plates on black tubercles; lateral fusiform areas distinct. Ortalidæ
   Stigmal plates barely if at all elevated; lateral fusiform areas indistinct; stigmal plates often contiguous or nearly so; slits long and subparallel..... Trypetidæ.
7. Slits in stigmal plates rather short, and arranged radiately..... 8
   Slits slender and subparallel to each other..... 9
8. Two tubercles above anal area; stigmal field with distinct processes around it..... Anthomyiidæ.
   Four or more tubercles above anal area; slits of stigmal plates usually pointed at one end..... Muscina.
9. A button to each stigmal plate; slits rather transverse to body.. Calliphorinæ.
   No button to stigmal plates, slits of one plate subparallel to those in opposite plate; plates at bottom of a pit..... Sarcophagidæ

CHAPTER XXI

THE MOSQUITOES

Mosquitoes (Culicidæ) are of the greatest importance medically, not only from their influence upon health in general by reason of interference with sleep and possibly from direct transmission of disease, but, more specifically, they are the only means by which it at present appears possible to bring about infection with such diseases as yellow fever, malaria, filariasis, and possibly dengue. In addition, many diseases of animals are transmitted by mosquitoes.

The Culicidæ differ from all other Diptera in having scales on their wings and generally on head, thorax, or abdomen.

To identify a mosquito, examine a wing and note the scales; also note the presence of two distinct fork cells and, in addition, that the costal vein passes completely around the border of the wing, making a sort of fringe with its scales. Mosquitoes undergo a complete metamorphosis, there developing from the egg a voracious, rapidly growing larva; next, a nongrowing, nonfeeding stage—the pupa or nymph. In this latter the head and thorax are combined in an oval body, from the back of which projects the siphon tubes; and tucked in ventrally is a small tail-like appendage.

The fully developed insect emerges from the pupa.

The Culicidæ belong to the suborder Nematocera. These have long articulated antennæ and include four families: Culicidæ, Chironomidæ, Simulidæ and Psychodidæ.

The principal mosquito-like, blood-sucking Diptera which are frequently mistaken for mosquitoes—none of which have scales on their wings—are the following:

1. **Chironomidæ** or Midges.—The blood-sucking species of Chironomidæ, which are found in most parts of the world, belong chiefly to the genus *Ceratopogon*. These midges are of very small size, about $\frac{1}{12}$ inch long, are able to get through netting and, usually being in swarms, they are exceedingly troublesome. The antennæ have thirteen joints and the wings are shorter than the abdomen and have only longitudinal veins. One of the midges, the "jejen" of Cuba, is a great scourge, its small size enabling it to enter eyes and nostrils. The larva of *Chironomus* is a red worm-like creature; the pupa has a tufted head.

2. **Simulidæ** or Buffalo Gnats.—These are small blood-thirsty insects only about ⅛ inch in length. The thorax is humped, the legs are short and the proboscis short and inconspicuous. The antennæ have 11 joints but are rather short. One species, the *S. damnosum*, known by the natives of Uganda as "Mbwa," is greatly dreaded; its bites causing swellings and sores. Sambon has considered *Simulium reptans* as the transmitting agent of pellagra.

3. **Psychodidæ** or Moth Midges.—These are small, hairy, slender midges, with long legs and a short proboscis. The antennæ are long, hairy and consist of 12 to 16

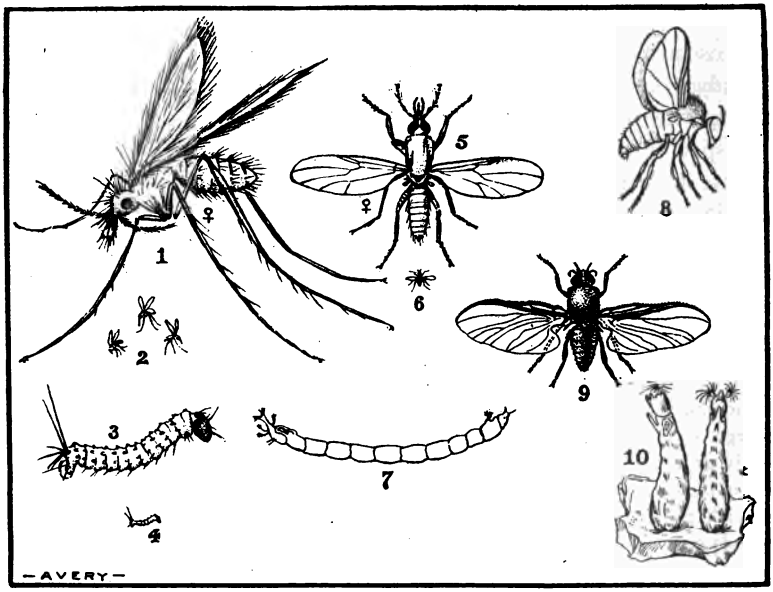


FIG. 113.—Mosquito-like insects belonging to families Chironomidæ, Simulidæ and Psychodidæ. (1) *Phlebotomus papatasii;* (2) *P. papatsii* (natural size); (3) *P. P. papatasii* (larva); (4) *P. papatasii* larva (natural size); (5) *Ceratopogon pulicaris;* (6) *C. pulicaris* (natural size); (7) *Chironomus* larva; (8) Attitude of a *Simulium;* (9) *Simulium reptans;* (10) Larvæ of *Simulium.*

joints. Palpi four jointed. They are only about 1/12 inch in length. The hairy wings have numerous longitudinal veins. Some, as *Phlebotomus*, have an elongated proboscis and are vicious blood suckers.

At present, of the genera of the three families of midges, only *Phlebotomus* is known to transmit disease. *P. papatasii* transmits phlebotomus fever in the Balkans. *P. minutus* is the host at Aden. Another species, *P. perniciosus* can transmit the disease. These moth midges are 2 mm. in length and have the body densely covered with long yellow hairs. The second longitudinal vein has three distinct branches. The antennæ have 16 constricted joints and the proboscis is as long as the head. The species of *Phlebotomus* are separated by slight variations in

wing venation, palpal lengths, etc., thus the second segment of palpi of *P. papatasii* is a little longer than the third one while with *P. perniciosus* these segnents are of equal lengths. In *P. minutus* the second segment is only half the length of the third. The insect lays about 40 eggs in damp dark places. The period of metamorphosis from egg to insect is about one or two months, according to temperature.

Phlebotomus larvæ die out in dry soil and very wet earth is unfavorable. Moderate moisture and protection from light seem necessary for their development. The remains of dead insects also seem to make good breeding places. It is in cracks of old damp brick or stone walls that the female most often deposits her eggs. Caves are also selected. Blood seems necessary for the fertilization of the eggs but lizard blood seems more common in the stomach of *P. minutus* than human blood. They have also been observed to feed on other reptilian bloods. The female insect has been kept alive in captivity up to forty-six days.

Culicidæ.—Mosquitoes have three main parts of the body—the head, the thorax, and the abdomen. On the head, the space behind the two compound eyes is called the frons, in front, and the occiput posteriorly.

The nape is back of the occiput. The bulbous prolongation of the frons which projects over the attachment of the proboscis is the clypeus. The clypeus is hairy in the *Culex;* scaly in *Stegomyia.* The proboscis is straight in all mosquitoes of importance medically. It consists of a fleshy, scaled, gutter-shaped portion beneath, known as the labium, which terminates in two hinge-joint processes—the labella. At the end of the labium is a thin membrane (Dutton's membrane). It is through this that filarial embryos are supposed to pass on their way from the interior of the labium to enter the person bitten. The labium may be considered as the sheath of a knife, holding and protecting the slender, blade-like penetrating organs. Lying in this groove we have, from above downward, the horseshoe-shaped labrum-epipharynx, the undersurface of which is open. This when closed by the underlying hypopharynx forms a tube through which the blood is sucked up by the mosquito. In the hypopharynx, which somewhat resembles a hypodermic needle, is a channel, the veneno-salivary duct. It is down this channel that the malarial sporozoite passes. There are two pairs of mandibles and two pairs of maxillæ on either side of the hypopharynx—the mandibles above and the maxillæ below. The serrations of the maxillæ are coarser than those of the mandibles. The sensory organs, the palps, lie on either side of and slightly above the proboscis. These are of the utmost importance in differentiating mosquitoes and must not be confused with the antennæ, which are attached above the palpi and at the sides of the clypeus. These antennæ are of importance in distinguishing the sex of the mosquito.

The thorax is largely made up of the mesothorax, at the posterior margin of which is a small, sharply defined piece, the scutellum; this may be smooth or trilobed. Underneath and posterior to the scutellum is the metanotum; the metanotum is bare in Anophelinæ and Culicinæ, has hairs in Dendromyinæ and scales in Joblotinæ.

There is a pair of wings attached to the posterior part of the mesothorax and, more posteriorly still, a pair of rudimentary wings (halteres) attached to the metanotum.

The wing venation is important. The costa shows as a stout rib or vein bordering the upper side of the wing and running around the apex and lower border.

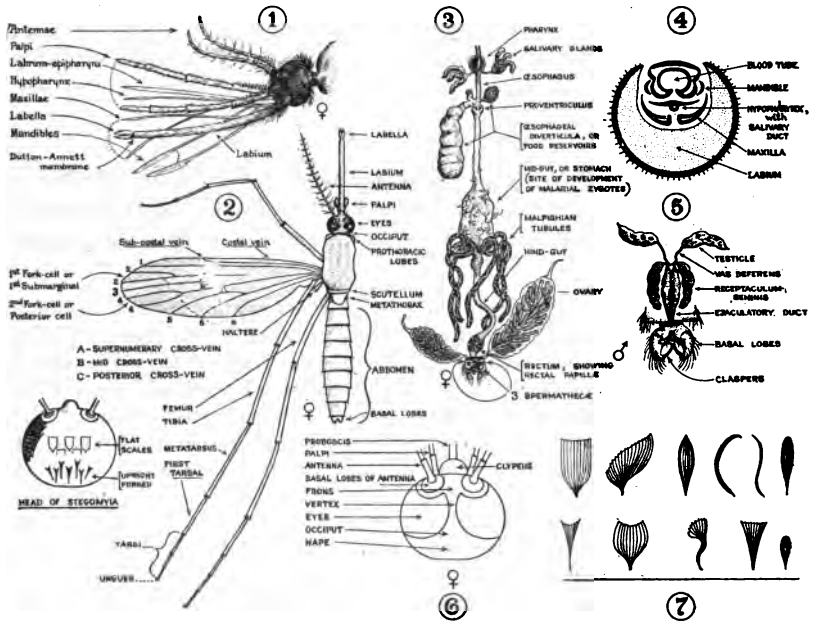


FIG. 114.—Anatomy of the mosquito. No. 7 shows various types of scales.

Below, it has a fringe which may show spots. The location of the spots in the upper part of the costa of anophelines is of great value in differentiating species. Beneath the upper costal border the subcostal vein runs to join the costa at some distance within the apex. The apex is the free end of the wing and the base that attached to the thorax. Running parallel to the subcosta, but reaching the apex, is the 1st longitudinal vein. Below that is the 2d longitudinal vein which forks to make the 1st fork cell or 1st submarginal cell. The 3d longitudinal takes origin at the junction of the supernumerary and midcross veins. The 4th longitudinal divides to form the 2d fork cell (2d posterior cell). The 5th and 6th longitudinal veins arise from the base of the wing and run to the periphery.

The three pairs of legs are attached to the thorax.

Each leg has 9 parts. The two short ones are the basally placed coxa and the

small trochanter attached to it. Then come the long femora, tibiæ and metatars" with the four segments of the tarsi terminally. The last tarsal segment ends in two claws, which in the female may be simple or uni-serrated.

There are nine segments in the abdomen. The genitalia arise from the terminal segments as bilobed processes. In the male there is a pair of hook-like appendages or claspers, between which, and ventrally situated, are the harpes, also a pair of chitinous processes.

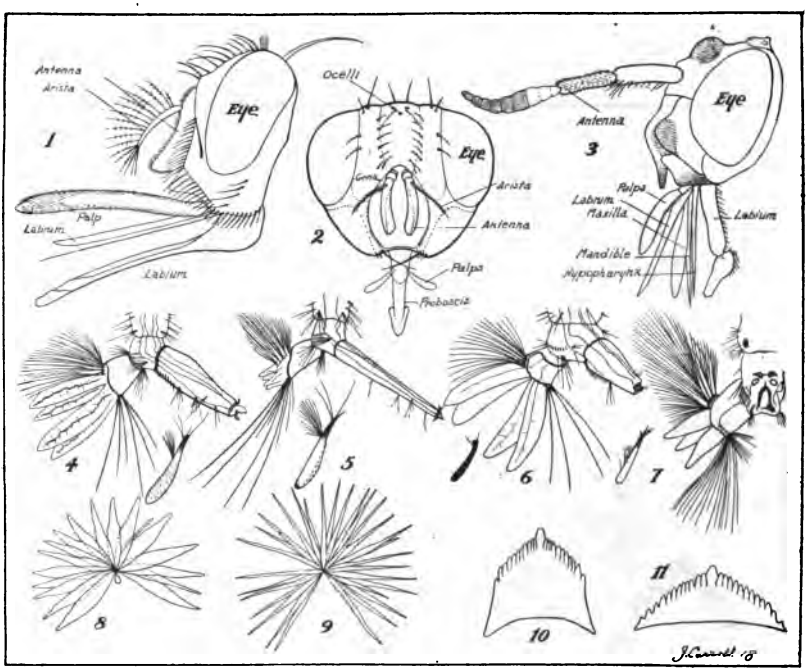


FIG. 115.—1. Head of *Glossina*. 2. Muscid fly. 3. *Chrysops*. 4. Syphon tube and antenna. *C. Quinquefasciatus*. 5. *C. Salinarius*. 6. *Aedes calo us*. 7. *A. punctipennis*. 8. Palmate hairs. *A. punctipennis*. 9. *A. albimanus*. 10. Mental plate. *C. pipiens*. 11. *A. calopus*.

In considering the question of the possible danger which might arise from the introduction of a case of yellow fever, malaria, or filariasis, it would give the greatest information if mosquito ova were at hand so that we could by watching the development from egg to larva, pupa, and insect, have all the points from which to decide as to the genera developing in the given locality. It is generally a very easy matter to dip out large numbers of larvæ from the pools and having noted the characteristics of the larvæ, to do the same when the pupæ develop; so that we have only to verify our identification when the insect emerges from the pupa.

The Ova

The egg raft of *Culex*, containing about 250 ova, is quite perceptible on the surface of the water as a black, scooped-out mass, about ⅛ inch in length. The eggs are set vertically in the raft. The eggs of the *Stegomyia* are laid singly and have a pearl-necklace-like fringe around them.

The Anophelinæ eggs are oval in shape with air-cell projections from either side. They are laid in triangle and ribbon patterns. The markings of these air cells vary and have been used for differentiation. The length of time of the egg stage varies according to temperature and other conditions—one to three days for *Stegomyia* and two to four days for *Anophelinæ*. The Anophelinæ are more difficult to raise than *Culex* or *Stegomyia*.

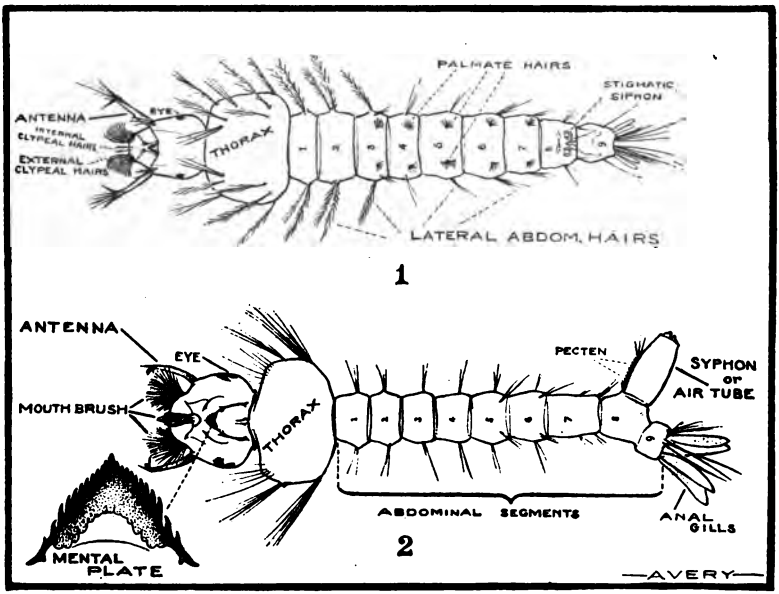


Fig. 116.—1. Asiphonate larva. *Anopheles*. 2. Siphonate larva. *Stegomyia*.

Larvæ

There are two great classes of larvæ—the siphonate and the asiphonate. The latter are always Anophelinæ.

The Culicinæ larvæ have a projecting breathing tube at the posterior extremity which is called a respiratory siphon. This projects off at an angle from the axis of the body, the true end of which terminates in four flap-like paddles. If you divide the length of the siphon by the breadth, you get what is known as the siphon index. In *Culex* the siphon is long and slender, in *Stegomyia* it is short and barrel-shaped. When at the surface the *Culex* larva has its siphon almost vertical and the body at an angle of about 45°.

The *Stegomyia* larva hangs more vertically. As a rule, the hairs proceeding from the sides of *Culex* larvæ are straight and the head relatively large. There are also no palmate hairs along the sides.

The Anophelinæ larvæ have a small head which is capable of being twisted around with lightning-like rapidity. They are darker in color and have no siphon; float parallel to the surface of the water; have long lateral branching hairs, and on the sides of each of the five or six middle abdominal segments they have a pair of palmate hairs. These palmate hairs are supposed to aid them in keeping their position on the surface of the water. The larvæ are usually called "wrigglers." The duration of the larval stage is from one to two weeks according to the temperature.

It has been proposed to use larval characteristics in differentiating species but as the larva moults about three times and as the hairs or spines of the exo-skeleton of these different larval stages vary in number and appearance such a scheme has not met with general approval.

The Pupæ

The pupa of the mosquito is an obtected one there being only a closely applied chitinous coating covering it; it does not have a puparium as does the coarctate pupa of the house fly. The mosquito pupa is lighter than water while the larva is heavier.

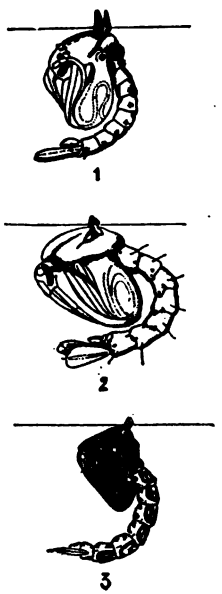


FIG. 117.—Pupæ: 1. *Culex;* 2. *Anopheles;* 3. *Aedes calopus.* (*After Howard.*) From P. H. Reports.

Pupæ have a bloated-looking cephalo-thorax and a shrimp-like tail—the latter the abdomen. Very important in examining them with a lens is to note the characteristics of the siphon tubes which project from the dorsal surface. These siphons are long and slender in *Culex* and project from the posterior portion of the head end. In Anophelinæ they are broadly funnel-shaped and arise from the middle of the head end. The siphon of the *Stegomyia* is triangular.

The bulbous end of the *Culex* nymph is more vertical than the horizontally placed cephalo-thorax of *Anopheles*. The duration of pupal life is short—only one to three days. At the end of this time the pupa comes to the surface and straightens out. The integument then splits dorsally and the perfect insect emerges. It dries its wings for a time on its raft-like pupal skin and then flies away.

From the above it will be seen that the stages in the metamorphosis of the mosquito take about two weeks: one to three days for egg stage; seven to ten days for larval stage and two to three days for pupal stage.

Dissection of the Mosquito

The easiest way to secure a mosquito for dissection is to use an ordinary plugged test-tube. Slipping the open end of the test-tube over the resting mosquito, by a slight movement, the insect will fly toward the bottom. Then quickly insert the plug. If it is not desired to study the scales, the best way to kill the mosquito is by striking the tube sharply against the thigh. If it is also desired to study the scale characteristics it is better to put a drop or so of chloroform on the lower part of the

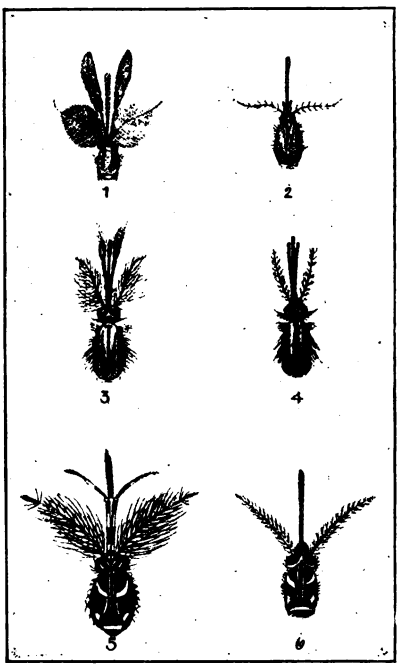


Fig. 118.—Heads of mosquitoes: 1 and 2, male and female *Culex pungens;* 3 and 4, male and female *Anopheles;* 5 and 6, male and female *Aedes calopus.* (*After Stitt.*) From P. H. Reports.

cotton plug. The vapor falls to the bottom of the tube and kills the mosquito. Take the mosquito out, pull off legs and wings, and then place the body in a drop of salt solution on a slide. It has been recommended to smear the surface of the slide with bile, wiping off the excess, before commencing the dissection in the salt solution. Then hold the anterior end of the thorax by pressure of a needle. With a second needle in the other hand, gently crush the chitinous connection between the sixth and seventh segments of the abdomen. Then holding the thorax firm, steadily and gently pull the last segments in the opposite direction. If this is done properly, a delicate gelatinous white mass will slowly float out in the salt solution. One should

be able to secure the alimentary canal as far up as the proventriculus, which is just anterior to the stomach, the part in which the malarial zygotes develop. Proceeding from before backward, we have the proventriculus, which is a sort of muscular ring at the opening of the stomach or mid-gut and marks the separation of the stomach from the œsophagus. Opening into the lower part of the œsophagus are the œsophageal diverticula or crops, which are food reservoirs. Occasionally in a dissection we pull out these structures which are three in number.

Leading from the stomach we have the hind-gut, which ends in the rectum.

This has a posterior dilatation or rectal pouch which usually has three or four rather marked anal papillæ.

Taking origin at the posterior end of the stomach and festooning the hind-gut are five longitudinal tubes—the Malpighian tubules. These are characterized by large granular-like cells with a prominent refractile nucleus. They are regarded as the renal structures. It is in these tubules that the embryo of the *Filaria immitis* of the dog develops. In the female mosquito, the parts withdrawn may seem to be largely made up of the white oval ovaries. These are connected with the spermathecæ, in which the spermatozoa are stored after fecundation by the male. In the male the testicles are quite distinct. Next to the examination of the stomach for zygotes, which appear as wart-like excrescences on its outer surface, the most important structures are the salivary glands, where the malarial sporozoites are found. The easiest way to dissect out the salivary glands is to press down firmly, but gently, on the anterior part of the thorax, and then with the shaft of a second needle, pressing on the head to gently draw the head away from the thorax, so that by this expression and traction movement you extract them with the head segment. They are very minute and are to be told by their exceedingly high refractile appearance. To examine for sporozoites cover the glands protruding from the neck with a cover-glass and search with one-sixth objective for narrow, curved bodies in the substance of the glands. If present try to smear out the glands between the cover-glass and slide by pushing the cover-glass along, then withdrawing the cover-glass, dry quickly and stain the smear on slide or cover-glass with Wright's stain.

The sporozoites are narrow falciform bodies about 12μ in length, with a central chromatin dot.

A matter about which there is dispute is as to whether the salivary glands communicate with the alimentary canal. Theobald states that there is no connection between them.

Differentiation of Culicinæ and Anophelinæ

It is impossible even for an entomologist to differentiate mosquitoes without recourse to elaborate keys and tables. It is a comparatively easy matter, however, to decide as to whether the mosquito is a probable malaria transmitter or not.

While certain characteristics of the male are used to separate the Ædinæ from other subfamilies, yet it is only with the female that we concern ourselves in differentiating the Culicinae from the Anophelinæ. Therefore, it is first necessary to dis-

tinguish the male from the female. If the antennæ have not been torn off, this can be decided by the highly adorned plumose antennæ of the male, those of the female being sparsely decorated with short hairs. The palpi of the Anophelinæ tend to be clubbed, while those of the *Culex* are straight. If the antennæ have been broken off, look for the claspers at the end of the abdomen.

Male mosquitoes do not feed on blood but on fruits and flowers instead. The puncturing parts of the male are not sufficiently resistant to penetrate the skin.

Having determined that the insect is a female, we then proceed to place it either in the subfamily Culicinæ or Anophelinæ by a study of the relative length of the palpi to the proboscis. If the palpi are shorter than the proboscis, it belongs to the Culicinæ; if as long or longer, to the Anophelinæ. The palpi of the female Megarhininæ are also long, but the proboscis is curved.

FIG. 119.—Resting posture of mosquitoes: 1 and 2, *Anopheles;* 3 *Culex pipiens.* (*After Sambon.*) From P. H. Reports.

Having settled on the subfamily, we separate the genera by considering such points as character and distribution of scales on back of head, wings, thorax, and abdomen; banding of proboscis, legs, abdomen, and thorax, shape of scales on wings, and location of cross veins.

Anophelinæ show abundant upright forked scales on occiput. The mesothorax shows sparse hairs or scales with a smooth scutellum. As a rule, the wings are spotted (dappled) and the location of these spots gives the best clue to the different species of the genera. With the exception of *Bironella* the first submarginal cell is large. This cell is longer than the second posterior one.

In the resting position Culex allows the abdomen to droop, so that it is parallel to the wall. The angle formed by the abdomen with head and proboscis gives a hunchback appearance.

Anopheles when resting on a wall goes out in a straight line at an angle of about 45°. It resembles a bradawl.

Classification

There are four subfamilies of Culicidæ, differentiated according to the palpi:

1. Palpi as long or longer than proboscis in male.
   1. Palpi as long as proboscis in females; proboscis straight. *Anophelinæ.*
   2. Palpi as long or longer than proboscis in females; proboscis curved. *Megarrhininæ.*
   3. Palpi shorter than proboscis in females. *Culicinæ.*
2. Palpi shorter than proboscis in male and female. *Ædinæ.*

The important ones from a medical standpoint are the Anophelinæ and Culicinæ.

Anophelinæ

1. Scales on head only; hairs on thorax and abdomen.
   1. Scales on wings, large and lanceolate. *Anopheles.* Palpi only slightly scaled.
   2. Wing scales small and narrow and lanceolate. *Myzomyia.* Only a few scales on palpi.
   3. Large inflated wing scales. *Cycloleppteron.*
2. Scales on head and thorax (narrow curve scales). Abdomen with hairs.
   1. Wing scales small and lanceolate. *Pyretophorus.*
3. Scales on head and thorax and abdomen. Palpi covered with thick scales.
   1. Abdominal scales only on ventral surface. Thoracic scales like hairs. *Myzorhynchus.* Palpi rather heavily scaled.
   2. Abdominal scales narrow, curved or spindle-shaped. Abdominal scales as tufts and dorsal patches. *Nyssorhynchus.*
   3. Abdomen almost completely covered with scales and also having lateral tufts. *Cellia.*
   4. Abdomen completely scaled. *Aldrichia.*

NOTE.—Of the above genera only *Cycloleppteron* and *Aldrichia* are unproven malarial transmitters.

The following species of anophelines selected from the different genera are important transmitters of malaria.

Anopheles maculipennis.—Wings with four spots located at bases of both forked cells and of second and third longitudinal veins. No costal spots. Palpi yellowish brown and unbanded. Legs unbanded.

Myzomyia funesta.—Wings with four yellow spots on a black costa and two black line spots on third longitudinal vein. Palps with three white rings. Proboscis unbanded. Legs with faint apical bands.

Pyretophorus costalis.—Costa black with five or six small yellow spots. Palps with two narrow white bands and white tip. Femora and tibiæ with yellow spots. Apical tarsal bands.

Myzorhynchus pseudopictus.—Black costa with two pale yellow spots. Wing fringe unspotted. Black palps with four pale bands. Apex of palps white.

Nyssorhynchus fuliginosus.—Black costa with three large yellow spots. Numerous black dots on the longitudinal veins. Palpi black with white tip and two narrow white bands. Last three hind tarsal segments white.

Cellia argyrotarsis.—Black costa with two distinct and several smaller white spots. Dark brown palps with two narrow bands and a white tip. Legs with last three hind tarsal segments white.

The **Megarhininæ** are of no importance medically.

The genus *Megarhinus* has the following characteristics:

1. Large mosquitoes with brilliant metallic coloring. (Elephant mosquitoes.)
2. Long, curved proboscis.
3. Caudal tufts of hairs on each side of abdomen.

The **Ædinæ** are not known to play any rôle in transmission of diseases. This subfamily is characterized by having the maxillary palpi much shorter in both males and females than the proboscis.

One genus *Sabethes* is very characteristic, owing to dense paddle-like scale structures on two or more legs.

Differentiation of Culicinæ Genera

1. Posterior cross vein nearer the base of the wing than the mid-cross vein.
   1. Proboscis curved in female. *Psorophora.*
   2. Proboscis straight in female.
      A. Palps with three segments in the female.
         (*a*) Third segment somewhat longer than the first two. *Culex.*
         (*b*) The three segments equal in length. *Stegomyia.*
      B. Palps with four segments in the female.
         (*a*) Palps shorter than the third of the proboscis. Spotted wings. *Theobaldia.*
         (*b*) Palps longer than the third of the proboscis. Irregular scales on wings. *Mansonia.*
      C. Palps with five segments in the female. *Tæniorhynchus.*
2. Posterior cross vein in line with mid-cross vein. *Joblotina.*
3. Posterior cross vein further from base of wing than mid-cross vein. *Mucidus.*

Of the Culicinæ the genus *Stegomyia* is of importance on account of yellow fever. The totally efficient hosts for filariasis (filarial embryos found in the thorax and proboscis) are chiefly among the genus *Culex.*

The genera *Mansonia* and *Tæniorhynchus* may also transmit filariasis. Some think the Anophelinæ genera *Cellia* and *Myzomyia* may transmit filariasis as well as malaria.

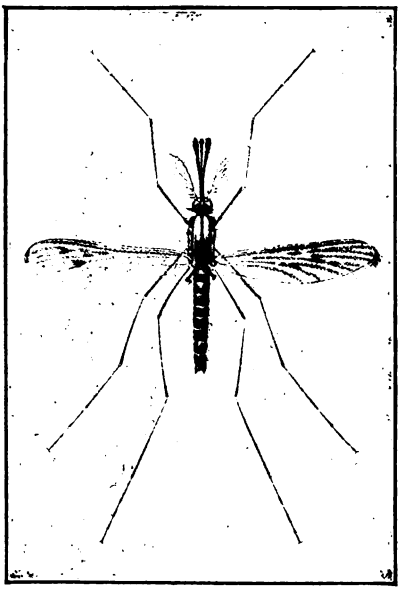


FIG 120.—*Anopheles maculipennis* (*quadrimaculatus*), *male.* (*After Castellani and Chalmers.*) From P. H. Reports.

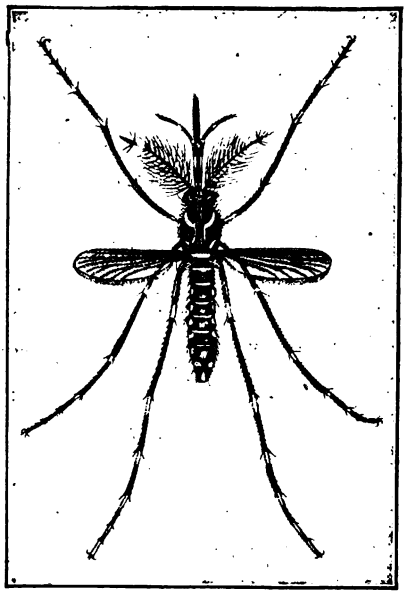


FIG. 121.—*Aëdes calopus*, male (*Stegomyia calopus*). From P. H. Reports.

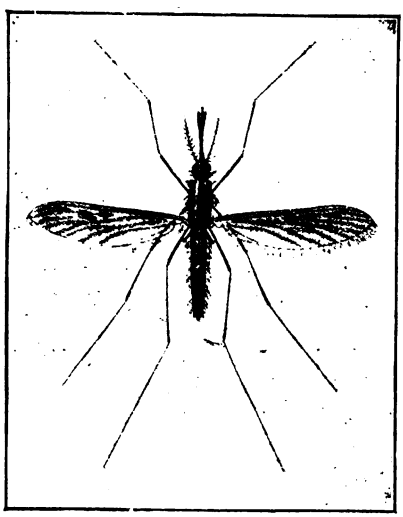


FIG. 122.—*Anopheles maculipennis* (*quadrimaculatus*), female. (*Castellani and Chalmers, after Austen.*) From P. H. Reports.

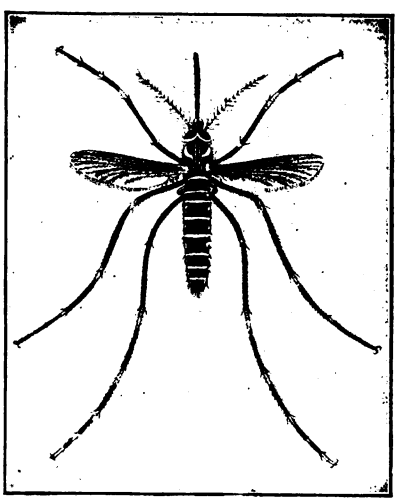


FIG. 123.—*Aedes calopus, female* (*Stegomyia calopus*). From P. H. Reports.

The genus *Culex* is implicated in dengue. Quite recently it has been demonstrated that *Stegomyia calopus* transmits dengue in Australia.

Stegomyia.—This is the most important culicine genus. These are mosquitoes with silver markings. The head, entirely covered with flat scales, has also some upright forked scales. Scutellum has dense flat scales. *S. calopus* is deep blackish-brown with two thoracic parallel lines with curved silver-white lines outside (lyre marking). Banding of thorax, abdomen, and legs. Howard gives this mosquito the name *Aëdes calopus.*

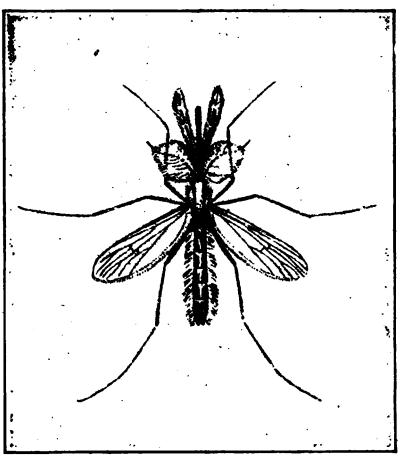


FIG. 124.—*Culex pungens*, male. (*After Howard.*) From P. H. Reports.

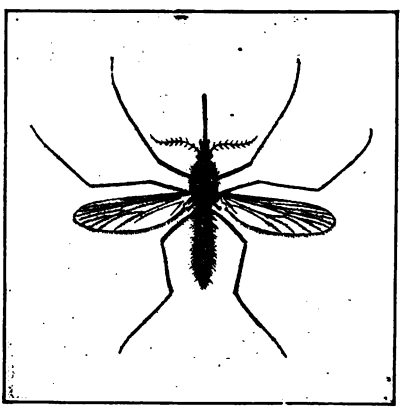


FIG. 125.—*Culex pungens*, female. (*After Howard.*) From P. H. Reports.

S. calopus bites only at night after the first feeding. The first meal of blood however may be taken in the daytime. To become infected it must take blood from a yellow-fever patient in the first two or three days of the disease. After sucking the blood of a yellow-fever patient the mosquitoes cannot transmit the disease by biting a nonimmune to yellow fever for a period of twelve days. After this time the mosquito remains infective for its life—in one instance fifty-seven days.

S. scutellaris has a single silver stripe down the center of thorax. Mosquitoes of this genus are often called "Tiger mosquitoes." The larvæ have short, barrel-shaped siphons. They breed particularly in receptacles about the house.

S. pseudoscutellaris, which resembles *S. scutellaris*, but has white bands only, at the sides of the abdominal segments, is thought to transmit filariasis in Fiji.

Culex.—Male palpi long and acuminate. Head has narrow curved and upright forked scales. Laterally, flat scales. *C. fatigans* supposed to carry dengue as well as *Filaria bancrofti.* It also transmits *Proteosoma* of birds, the life history of which in this mosquito paved the way to the epochal discoveries in connection with malarial

transmission by anophelines. This is a brown mosquito with pale yellow banding of each abdominal segment. The legs are brown except for the coxæ and femora.

Theobaldia.—These Culicinæ have spotted wings resembling Anophelinæ. These spots are due to aggregations of scales, not to dark scales. Male palps are clubbed (like *Anopheles*).

Mucidus.—This genus has a mouldy look from long twisted gray scales. The legs are densely scaled.

Mansonia.—This genus is characterized by broad flat asymmetrical wing scales. As the wing scales are brown and yellow the wings are mottled.

Grabhamia.—Wings have pepper-and-salt appearance with short fork cells.

Tæniorhynchus.—This genus is characterized by dense wing scales, which are broadly elongated with truncated apex.

Acartomyia.—Much like *Grabhamia*, but scales of head give ragged appearance. Male palpi clubbed.

A. zammittii was supposed to be concerned in Malta fever, but it is known now that transmission is by medium of milk of infected goats.

CHAPTER XXII

POISONOUS SNAKES

SNAKES belong to the class Reptilia and the order Ophidia. The two families to which poisonous snakes belong are the colubrine snakes (Colubridæ) and viperine snakes (Viperidæ).

Colubrine Snakes.—Of the Colubridæ the Hydrophinæ or sea-snakes with rudder-like compressed tail and the Elapinæ with round tails are most important.

Many of our harmless snakes such as the garter-snake and blacksnake belong to the Colubridæ.

The cobras belong to the subfamily Elapinæ and are best known by a neck-like expansion or hood. The only poisonous colubrine snakes in the United States are the beadsnake (*Elaps fulvius*) often called the Florida coral snake, and the sonoran coral (*Elaps euryxanthus*).

The beadsnake is black with about seventeen broad crimson bands, which bands are bordered with yellow.

Although small, they are very venomous. The upper jaw has anteriorly grooved fangs, which appendages are not present in the nonpoisonous coral snakes, these latter having teeth in the upper jaw so that the wound shows four rows of punctures instead of two rows and one larger puncture on each side to mark the entrance of the fangs.

In Asia there are many important poisonous colubrine snakes; the cobra (*Naja tripudians*), the King cobra (*Naja bungarus*) and the Kraits (*Bungarus fasciatus*).

All of the Australian poisonous snakes are colubrines.

Viperine Snakes.—The Viperidæ which are characterized by a triangular head and tubular poison fangs are the most important poisonous snakes in America. The rattlesnake (*Crotalus*), the copperhead (*Ancistrodon contortrix*), and the water moccasin (*A. piscivorus*) are widely distributed in the United States.

There are many harmless snakes which more or less resemble these "Pit Vipers" as the rattlers, moccasins, and copperheads are called. This term refers to a deep hole or pit found on the side of the head between the nostril and the eye. It is a blind sac.

Some divide the Viperidæ into the Crotalinæ, which possess the pit and the Viperinæ which do not have this structure. Russell's viper (*Vipera russellii*) is the best known of the Viperinæ and is one of the important poisonous snakes of India.

The poison fangs are grooved or perforated and connected with the poison glands which resemble salivary glands and may be almost an inch in length in large snakes. The tongue is slender and forked and is a tactile organ.

The jaws are remarkable for their great extensibility, not only vertically, but laterally, by the ligamentous connections of the two halves of the mandible or lower jaw.

As the fangs are directed backward it is necessary for the snake when striking to open widely the jaws and bend back the neck. The fangs are then brought forward and erected by the spheno-pterygoid muscles. The snake bite is a combination of bite and blow. The functional fangs of colubrine snakes however are not mobile.

In addition to the possession of the pit, these vipers have a more or less triangular head and in particular a single row of large scales on the undersurface posterior to the vent (anus), while the harmless snakes show an elongated oval head and two rows of large ventral scales posterior to the vent.

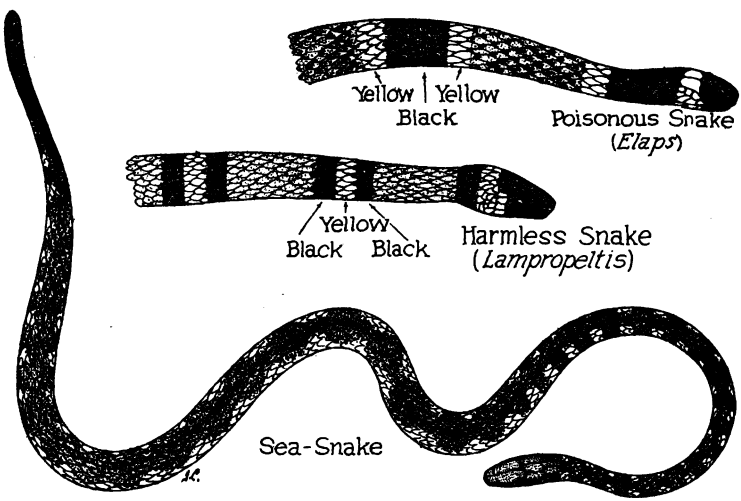


FIG. 126.—The poisonous coral snakes of the U. S., *Elaps fulvius* and *Elaps euryxanthus*, have transverse rings of black, vermillion and yellow. As differentiating these snakes from harmless ones which resemble them there are yellow rings bordered by two black ones, while with the harmless snakes a yellow ring is bordered by two black ones. The sea snake (Enhydrinæ species) has a rudder-like tail which is here shown twisted to one side.

Snake Venom.—In examining the wound made by a snake the two punctures of the fangs indicate the bite of a poisonous snake. If these fang puncture points are far apart it shows that a large snake, and probably one capable of injecting a greater amount of venom has given the bite.

When a snake strikes the fangs move from the horizontal to the erect position, the mouth being widely open. When the fangs enter the jaws close and pressure is exerted on the poison glands so that the venom pours out.

The amount of venom varies with the size and condition of the snake, an adult cobra yielding about 1 c.c. Acton and Knowles give the following table expressed in milligrams of desiccated venom.

Common cobra (mean yield)	317.0 mgm.
Common krait (mean yield)	8.17 mgm.
Banded krait (mean yield)	64.4 mgm.
Russell's viper (mean yield)	108.0 mgm.

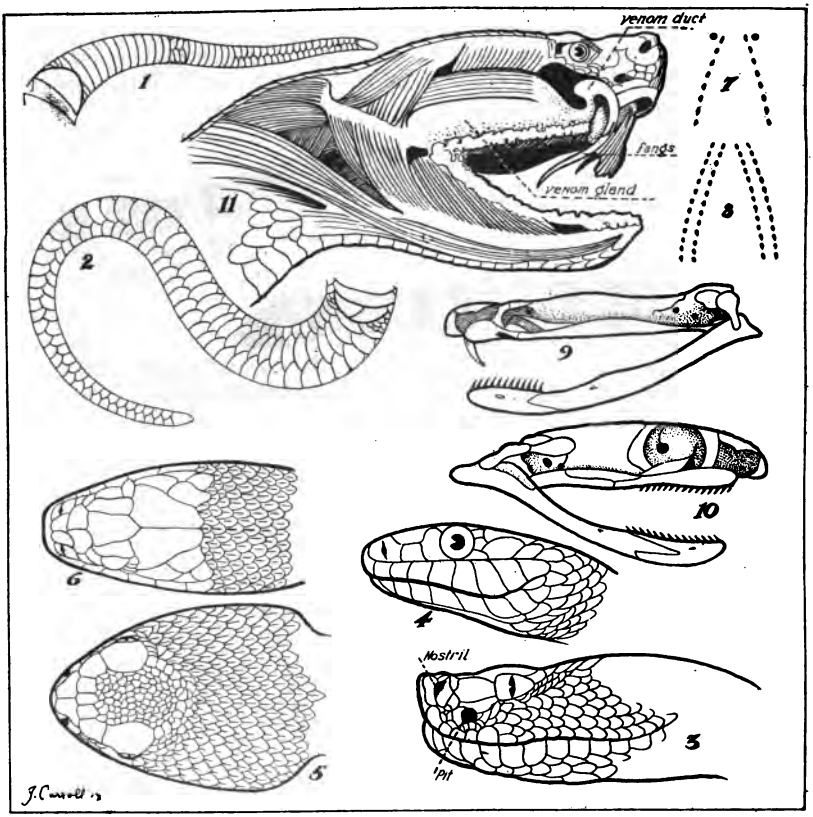


FIG. 127.—1, Single row of scales posterior to vent (poisonous snake—water moccasin); 2, Double row of scales of harmless snake (*Natrix*); 3, side view head of pit viper; 4, side view head of harmless snake; 5, dorsal view pit viper; 6, dorsal view of harmless snake; 7 and 9, bite puncture and skull of *Elaps;* 8 and 10, same of harmless snake; 11, poison apparatus of rattlesnake.

They estimate the minimum lethal dose for man as 15 mgm. with cobra venom and 42 mgm. with the venom of Russell's viper. The venom of the Kraits is more potent, that of the very common Indian krait, *Bungarus candidus* being given as 1 mg.

The cobra, after having bitten, remains attached for a short time while the daboia strikes with the greatest rapidity and immediately releases itself.

Cobra and krait bites (colubrine snakes) produce more or less similar symptoms such as paralysis of articulation with nausea and vomiting and later paralysis of the respiratory apparatus. There is only an insignificant reaction at the point of bite.

The venom is mainly neurotoxic, causing death by paralysis of cardiac and respiratory centers. Cobra venom is also very hæmolytic. This hæmolysin is acti-

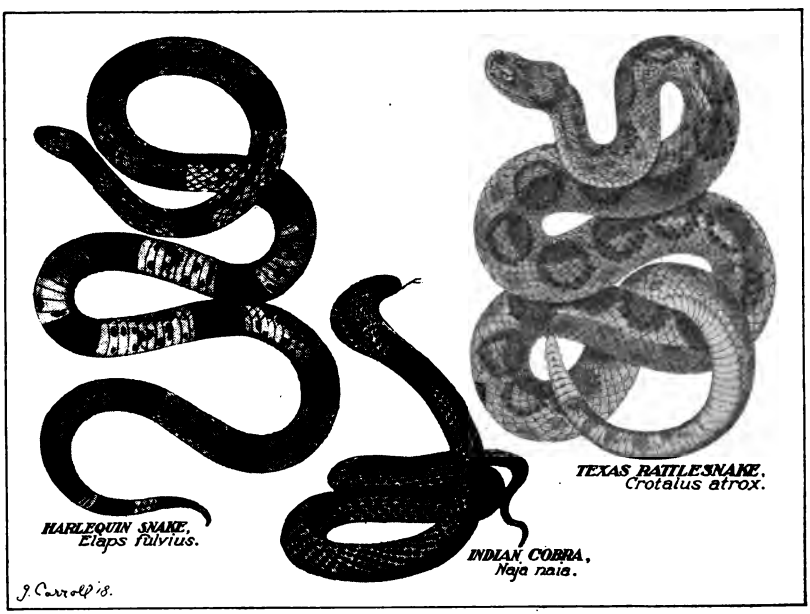


FIG. 128.—Important poisonous snakes.

vated by the normal complement of the serum of the animal poisoned, the hæmolysin as contained in the venom not being toxic when alone. Lecithin also has the property of activating the hæmolytic amboceptor of venom.

In rattlesnake bites (viperine snakes) there is marked pain at the site of the wound with much swelling and hæmorrhagic infiltration. The swelling and petechial mottling spread up the limb from the point of entrance of the venom. Cold sweats, nausea, weak heart, and syncope are common.

Rattlesnake venom is active chiefly on account of its hæmorrhagin or rather endotheliolysin, which destroys the endothelial lining of blood-vessels.

Venoms may also contain proteolytic ferments which may account for the softening of muscles in snake-bite cases. The toxic effect of the venom takes place without an appreciable incubation period, hence different from true toxins.

The most venomous snakes seem to be the sea-snakes (*Enhydrina*). This venom is almost entirely neurotoxic.

The tiger snake of Australia is almost equally venomous and the krait (*B. cæruleus*) next. The rattlesnake is about one-fifth as venomous as the krait.

Certain venoms greatly increase the coagulability of the blood so that intravascular thromboses may occur. It is chiefly with the venoms of *Daboia* and *Bungarus* that such thromboses are likely to occur and this accounts for the almost instantaneous death which at times results from bites of such snakes.

Treatment.—The nonspecific treatment of snake-bite poisoning is 1. by applying a tight ligature above the site of the bite. The ligature, which should preferably be a rubber band, is to be applied about a single bone extremity, not about one with two supporting bones. 2. The making of deep incisions about the fang punctures and thorough irrigation with a strong solution of potassium permanganate. Rogers has recommended that the punctures be enlarged with a lancet and the resulting wound packed with crystals of permanganate.

Recently Bannerman has shown that a dog bitten by a cobra cannot be saved by free incision and the rubbing in of permanganate crystals. It may however be saved by the immediate injection of 10 c.c. of a 5% solution of permanganate, but not if two minutes has elapsed. Bites from the daboia are fatal, however the permanganate be applied.

He therefore does not consider the permanganate treatment of any practical value. Rogers thinks that Bannermann's experiments with dogs do not give a true idea of the value of permanganate because he has had success in experimenting with cats and because it has saved human lives. Chromic acid injections (1%) have also been recommended. Acton and Knowles consider potassium permanganate as unreliable and recommend subcutaneous injections of a 5% solution of gold chloride. These local injections are efficacious if used before the venom has been absorbed but it must be understood that they have no effect on venom taken up by the circulation. Intravenous injection of permanganate is not only without effect but s dangerous.

Internally alcohol does not seem to be of any value, in fact many of the deaths have been attributed to excessive ingestion of whiskey. Strychnine in large, almost poisonous doses, was highly recommended in Australia but the statistics seem to make the value of this remedy doubtful.

Antivenins.—The active agents of snake venoms may be either of the nature of hæmorrhagins, neurotoxins, or fibrin ferments. In colubrine snakes the neurotoxin vastly predominates while with the viperines it is the hæmorrhagin. Certain Australian snakes contain all three bodies in about equal proportion while with the rattlesnakes of America it is almost entirely the hæmorrhagin which causes the poisoning. The Elaps of Florida is a colubrine snake and its venom is neurotoxic in nature.

The cause of death in colubrine snake bites is chiefly from paralysis of the respiratory centers while with the Pit Vipers it is chiefly from hæmorrhages in the vital

organs. Antitoxins have been prepared against both viperine and colubrine venoms and these are specific, thus a colubrine antivenin will not be of value against a viperine bite. Antivenins should be administered either intravenously or intramuscularly. The amounts recommended for injections to neutralize a fatal dose of snake poison vary from 100 to 300 c.c. of the antivenin serum. There is no accurate standardization.

Acton and Knowles recommend the following treatment for snake-bite:

(1) Apply a firm ligature immediately.

(2) Impregnate the whole area of the bite with a hypodermic injection of a strong solution of gold chloride.

(3) Inject from 100 to 200 c.c. of antivenene intravenously, if the biting snake be suspected to have been a cobra or Russell's viper. If symptoms of venom intoxication come on, further and even larger injections of antivenin should be given intravenously.

With sera concentrated 10 times, a dose of 20–60 c.c. should save every case of cobra bite.

CHAPTER XXIII

POISONOUS ARTHROPODS, FISH AND CŒLENTERATES

Venomous Arthropods

Spiders.—Spiders belong to the class Arachnida, Order, Araneida. There are numerous families, divided into various genera. As a rule spiders secrete a venom which is capable of poisoning the small animals used as food but it is only in rare instances that the venom is poisonous for man. It must be remembered that individual idiosyncrasies make one person susceptible to spider or other arthropod bites while others do not suffer.

Reports of illness following spider bites are very rare and many of these are due to secondary infections with pyogenic bacteria.

The dread of spiders is probably connected with the attributing of the hysteria of the Middle Ages or tarantism, to the bite of *Lycosa tarantula*. As a matter of fact the bite of this spider only produces a localized erythema without general symptoms.

Experiments have shown that most of the common spiders not only are unwilling to bite but, even when almost forced to do so, are unable to penetrate other than the most delicate human skin. Even then the bite has only the effect of a pin prick.

In America we apply the term tarantula to a large, dark, hairy ferocious-looking spiders of the family of Aviculariidæ. The American tarantula, *Eurypelma hentzii*, is capable of killing very small animals but it is only believed to be able to inflict mechanical injury on man.

Certain species of the genus *Latrodectus* produce systemic symptoms rather than local ones. The bite of *L. scelio*, of New Zealand, the "Katipo" spider, is stated to slow the pulse and respiration together with production of tetanoid manifestations. In the U. S., a venomous spider, *L. mactans*, which is black, with one or more red spots on the dorsal abdomen, can produce more or less serious symptoms and possibly, but certainly most exceptionally, fatal results in man.

Scorpions.—These arachnids belong to the order Scorpionida. The scorpions of temperate climates are usually small but in the tropics they may attain very large size, even 7 inches in length. The last abdominal segment terminates in a ventrally curved spine. This segment carries the poison glands.

Scorpions have formidable claws or pedipalps, with which they seize their prey and then by a downward movement of the tail-like abdomen they pierce the prey with the spine and thus introduce their venom. The poison of some of the large scorpions, as *Buthus quinquestriatus*, seems to resemble in action that of the cobra venom. While the larger scorpions are particularly to be dreaded and especially where young children have been bitten (mortality of bites of *B. quinquestriatus* in young children practically 50%) the effects of the bites of the small scorpions found in the Southern U. S. and California are probably never fatal but may be quite painful and produce slight general symptoms.

Myriapods.—These arthropods are divided into the orders, Diplopoda, or millipedes and Chilopoda, or centipedes. Millipedes have a more cylindrical body than centipedes and with the exception

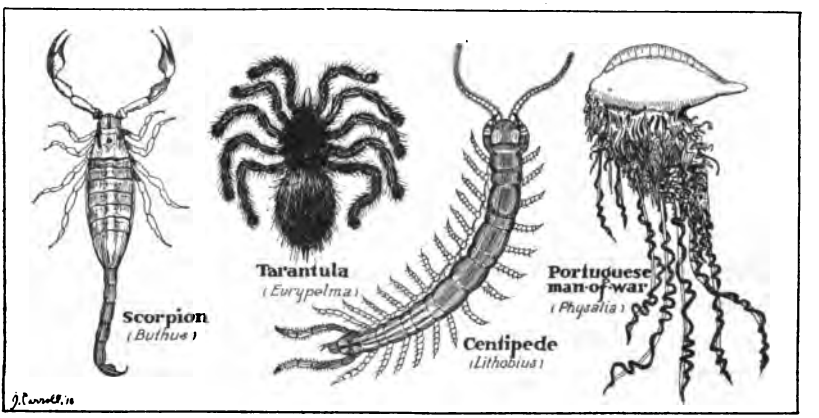


FIG. 129.—Poisonous arthropods and cœlenterates.

of the appendages coming from the most anterior somites, have two pairs of legs to each segment, while the centipedes have only one pair to each segment.

It is generally accepted that millipedes are harmless. Centipedes have poison glands at the base of the first pair of legs, which legs terminate in a powerful claw, at the tip of which is the opening for the expulsion of the venom. The small centipedes which are found in temperate climates rarely give rise to more than local symptoms but the large tropical ones, as for instance *Scolopendra gigantea*, which may be 10 or 12 inches long, may cause death in children by their bite and give rise to necrotic local lesions at the sites of the two punctures and, in addition, may produce general symptoms of vomiting, headache, fever and even coma.

Bees, Wasps and Ants.—These arthropods belong to the order Hymenoptera of the class Insecta. The venom of bees is ejected

through the sting which is at the end of the abdomen. In addition to the formic acid there is also a neurotoxin in the venom. When a bee stings the biting parts are left in the wound to continue by muscular action to force out the contained venom into the tissues of the victim.

As a rule the effects of a bee sting are entirely local but cases have been reported of general symptoms ensuing, such as fever, dizziness, dyspnœa and urticarial lesions.

The bumble bee differs from the honey bee in that the sting is not cast off when stinging. Hornets and wasps have a well developed sting and are more dreaded for their sting effect than bees.

Ants.—In temperate regions ants rarely are considered as producing injury but in the tropics there are large formidable species which may not only cause local irritation but even produce general symptoms of nervous system involvement.

In the Philippines the ants are prominent factors in destroying house fly larvæ so that in this way they are of great assistance to man.

Poisonous Fish

Fish Poisonous as Food.—The matter of illness produced by eating decomposed fish, whether in the natural state or canned, belongs to the general problem of food poisoning. There are, however, certain fish whose meat is poisonous when eaten in a perfectly fresh state.

This may be connected with certain epidemic diseases among fish ordinarily good food. Various bacterial organisms have been isolated from such fish and the poisonous effects have been attributed to various ptomaines elaborated by these toxicogenic organisms. Most of the organisms isolated from diseased fish have belonged to the colon or proteus groups. Cases have been reported of botulism like poisoning arising from the eating of insufficiently salted fish. These cases were probably due to the development of a soluble toxin by *B. botulinus*, as such fish when cooked lost their toxicity. It will be remembered that the toxin of *B. botulinus* is destroyed by heat, whereas that due to the Gärtner, or ordinary food poisoning organism, withstands ordinary cooking temperatures. This fish poisoning by bacterial products is designated *ichthyotoxismus*.

The only two animal parasite infections with which the eating of fish is connected are: (1) *Bothriocephalus latus* and (2) *Clonorchis epidemicus*. The broad Russian tape-worm is a rather common parasite of man in the Baltic provinces and comes from eating of insufficiently salted pike and other fish infected with this larval tape-worm. The

liver fluke disease of China and Japan is caused by the eating of various raw or insufficiently cooked fresh water fish. These fish are the secondary intermediate hosts, the primary one being molluscs.

There are certain fish whose meat is poisonous when there is no question of decomposition or disease in the fish. The best known instance is with certain species of the genus *Tetrodon*. The illness produced by the eating of this fish is usually termed *fuguismus*, the Japanese designating such fish by the term "fugu." The poisonous principles seem to exist chiefly in the ovaries and testicles, the eating of

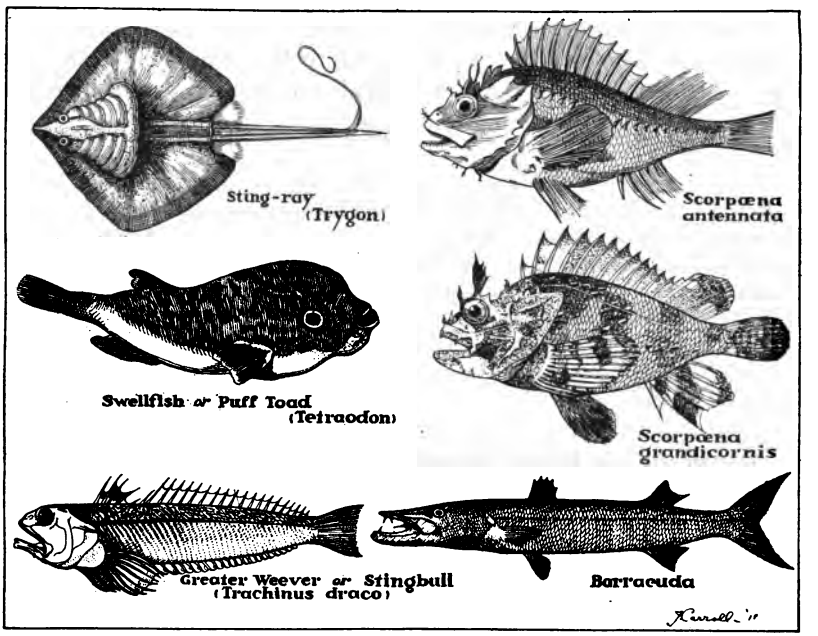


FIG. 130.—Poisonous fishes.

even one roe of such fish bringing on serious illness in a few minutes or possibly death in a few hours. It has been stated that after careful removal of all genital and alimentary tract organs these fish may be eaten without harm. The porcupine fishes or Diodontidæ are considered as poisonous. These fishes together with the Tetraodontidæ, or broad-nosed puffers, are unsightly in appearance. Among seamen they are generally designated puff toads as they become distended with air as they are drawn out of the water. It is well recognized that certain of these fish which may fail to cause poisoning at one time may do so at another time and it is particularly noted that poisoning effects occur at the time of spawning.

In the tropics fish which may ordinarily be safe as food become poisonous as a result of feeding on certain poisonous medusæ and corals.

There are certain species of the herring family which have a bad reputation. Among these are two species of *Meletta*. In New Caledonia, *M. venenosa* causes painful cramps of the body with dyspnœa, cyanosis, cold sweats and dilated pupils and at times death. *M. theissa* of the West Indies is also a very poisonous fish.

Fish Which Poison by Their Sting or Bite.—Fish of the genus *Muræna* have well developed teeth which are in relation to a poison sac which secretes a venom which is introduced into the wound made by the bite. There are also various rays which are well known all over the world as capable of inflicting wounds.

In the sting rays (Trygonidæ) the tail is armed on the upper side with a barbed spine which in some species is connected with a poison apparatus and in addition to the wound made inoculates a venom. Some of these sting rays when wounding a person who may step on them while wading in the water may at the same time inoculate tetanus bacilli which flourish in the deep punctured wound. In the electric rays (Torpedinidæ) the dorsal surface is electrically positive and the ventral one negative. To receive a shock one must communicate with the *Torpedo* species at two distinct points. Some of these electric rays are capable of temporarily paralyzing the arm of a man.

Two of the best known poisonous fish are *Trachinus draco* and *Scorpæna scropha*. The flesh of these fish is wholesome as food. *T. draco* is like a trout in appearance and has blue and brown stripes. It has a grooved spine passing through each of its gill covers which is connected with a poison gland. There is also a poison apparatus connected with the dorsal fin. *S. scropha* is an ugly red fish with large head and prominent fins. The French fisherman call it "le diable." The poison apparatus is connected with the first three rays of the dorsal fin.

Persons in bathing who strike against these fins are more apt to be wounded than the fishermen who proceed to handle the fish with caution. Following the wounding a person experiences stabbing pains of the affected part. A sensation of suffocation follows and the victim may become delirious. At times collapse and death result. At the site of the bite we first have an erythematous area which later becomes black and may become gangrenous. As the poison rapidly enters the general circulation treatment similar to the local treatment of a snake bite is called for. These fish seem to be more dangerous during the spawning period.

Poisonous Cœlenterates

In this branch of animals the most important ones from a standpoint of medicine are the anemones and jelly fishes. A condition known as "la maladie des plongeurs" occurs among the sponge fishermen of the Mediterranean. This is due to stinging by anemones and is characterized by marked itching, burning and erythema. In some cases the skin of the affected area becomes necrotic and sloughs off leaving an ulcer.

As a rule jelly fishes are harmless but certain species produce unpleasant or even serious effects by their sting.

In the Mediterranean a jelly fish *Rhizostoma pulini* produces œdema and urticarial eruptions as the result of its sting. In many parts of the tropics jelly fishes are found which give rise to quite serious symptoms. In the Philippines there are certain species of jelly fishes which cause serious illness, although as a rule one experiences no discomfort from coming in contact with many other species while swimming in the waters of that part of the world.

According to Light, the species of *Dactylometra*, called "fosforo" by the natives, is the most dangerous one there encountered. It has long ribbon-like oral lappets and 24 slender white marginal tentacles. In this the sting is inflicted by nematocyst batteries in the four long ribbon-like oral palps. *Lobonema*, called by the natives "lanterna," is of large size, white or white and purple in color and stings by the long filaments which arise from the mouth arms. I have treated a number of cases of jelly fish stinging in the Philippines which presented symptoms ranging from a mild erythema to those showing marked congestion of the respiratory tract and other general symptoms.

Old has described these symptoms very accurately and notes the following:

The symptoms appear in from ten to sixty minutes with marked hysterical manifestations, incessant cough and coryzal signs. Light believes that the cases described by Old were due to stinging by *Dactylometra*.

The Portuguese man of war (*Physalia*) has long locomotive tentacles which stretch out from 30 to 50 feet as the animal is blown along by its pearly purple crested bladder-like float or sail. The thread cells are capable of inflicting rather painful stings when handled without a knowledge of the effect of coming in contact with these thread cells.

PART IV

CLINICAL BACTERIOLOGY AND ANIMAL PARASITOLOGY OF THE VARIOUS BODY FLUIDS AND ORGANS

CHAPTER XXIV

DIAGNOSIS OF INFECTIONS OF THE OCULAR REGION

IT is advisable before taking material for cultures or smears to cleanse the nasal area of the eye-lids, and especially about the caruncles, with sterile salt solution. Then, by gently pressing on the lids, we may be able to get pure cultures of the organism causing the infection. Normally, we may find in the region of the caruncles various skin organisms, especially staphylococci, giving white colonies.

The xerosis bacillus and white staphylococci may be considered normal findings in the conjunctival sac. Streptococci and pneumococci have also been reported from apparently normal conjunctival secretions.

A small particle of sterile cotton, wound on a toothpick, with the aid of a sterile forceps, makes an excellent swab for obtaining material for smears; the same may first be drawn over an agar surface in a Petri dish in a series of parallel lines of inoculation before making the smears on slide or cover-glass.

When there is a considerable discharge, a capillary pipette, with a rubber bulb, may be used to draw up sufficient material for cultures and smears. Be sure to round off the end of the pipette in the flame and not to use a very fine capillary tube.

In conjunctival cultures, plates of glycerine agar, blood agar, or agar plates smeared with blood are to be preferred, as the *Gonococcus* and Koch-Weeks bacillus will only grow on blood or hydrocele agar. The diphtheria and xerosis bacilli grow well on glycerine agar.

In addition to the white *Staphylococcus*, the *Streptococcus* may be present when inflammation of the nasal duct exists.

. The *Streptococcus* is at times responsible for a pseudomembranous conjunctivitis. The *Staphylococcus* is as a rule the cause of phlyctenular conjunctivitis.

The *Pneumococcus* is a fairly common cause of serpiginous corneal ulcerations. Active treatment is necessary.

It is now recognized as advisable to make an examination for the *Pneumococcus* before performing operations on the eye as serious results may follow if the *Pneumococcus* be present. It is the organism frequently found in dacryocystitis and, in the case of traumatism, may bring about panophthalmitis.

Corneal ulcerations are not apt to appear even with a pneumococcal conjunctivitis unless there be an injury of the epithelium.

The *B. xerosis* is possibly a harmless organism and must not be accepted as explaining an infection unless other factors have been eliminated. The true diphtheria bacillus, which the xerosis so much resembles, may cause a pseudomembranous inflammation.

The *B. pyocyaneus* may cause severe purulent keratitis as well as conjunctivitis. The pyocyaneus toxin appears to be a factor.

The *Gonococcus* and the Koch-Weeks bacillus are usually responsible for the very acute cases of conjunctivitis. Both these organisms are characteristically intracellular and are Gram negative.

Conjunctivitis in the course of epidemic cerebrospinal meningitis has been found to be due to the *Meningococcus*. It may even cause panophthalmitis.

The diplobacillus of Morax and Axenfeld is more common in chronic, rather dry affections of the conjunctiva, chiefly involving the internal angle and showing a morning accumulation of the secretion. The bacilli are found in twos, more rarely in short chains. They are generally free but may be found in phagocytic cells. They resemble Friedländer's bacillus morphologically but do not have capsules.

In cases of ozena with involvement of the nasal ducts Friedländer's bacillus may be found.

Even in cases without ozena, capsulated, Gram-negative bacilli of the Friedländer group have been frequently reported in conjunctival inflammation and in dacryocystitis as well.

The nodules of the eye-brows give the most convenient area to take material from in the diagnosis of leprosy, either the fluid expressed after scraping or a piece of tissue cut into sections. Conjunctival ulceration in leprosy may show abundant bacilli as is also true of corneal ulceration.

Ordinarily it is impossible to find tubercle bacilli in tuberculous conjunctival discharges.

The discharge from a tuberculous dacryocystitis may show them satisfactorily. Animal inoculation is preferable in the diagnosis of ocular T. B. The *Pneumococcus* is, however, the most important organism in dacryocystitis—rarely the *B. coli.*

In a gonorrhœal ophthalmia the secretion is much more abundant and there is an absence of contaminating organisms, the reverse of infection with the confusing *M. catarrhalis.* As a matter of fact, large numbers of *M. catarrhalis* may be present in the conjunctival secretion with only slight irritation being observable.

Wherry has reported two cases of ulcerative conjunctivitis with lymphadenitis of cervical glands, fever and marked prostration, due to infection with *B. tularense,* occurring in persons who had handled rabbits which had died of this plague-like infection. The organism was first noted by McCoy in squirrels in California. The symptoms and lesions in these animals are those of plague. Guinea-pigs succumb after the cutaneous inoculation of material and show lesions markedly resembling plague. The organism, however, will only grow on coagulated egg yolk, thus differentiating it from *B. pestis.* McCoy has noted that the infection in squirrels may be transmitted by fleas (*Ceratophyllus acutus*).

In keratomycosis the cause has been ascribed to *Aspergillus fumigatus.*

Certain fungi of the genus *Microsporum* have been thought to be the cause of trachoma, as have also certain bacillary forms. One should be very conservative about reporting fungi in smears or cultures of external surfaces.

The larval stage of *Tænia solium* (*Cysticercus cellulosæ*) has a predilection for eye as well as brain. It is usually situated beneath the retina.

The question as to the nature of the so-called ophthalmic flukes is taken up under trematodes. *Echinococcus* cysts have been reported in the orbit.

The adult *Filaria loa* tends at times to appear under the conjunctiva or in the subcutaneous tissue of the eye-lids.

Fly larvæ have been reported from the conjunctival sacs in the helpless sick, species of larval sarcophagids having been reported as invading the conjunctival region in purulent ophthalmias.

Demodex may cause an obstinate blepharitis.

Prowazek has thought that certain fine dots within the cytoplasm of epithelial cells, which stain best by Giemsa's method and which he considered protozoal in nature, were the cause of trachoma. See Koch-Weeks bacillus and trachoma bodies.

CHAPTER XXV

DIAGNOSIS OF INFECTIONS OF THE NASAL AND AURAL CAVITIES

In taking material from the nasal cavities, for bacteriological examination, it is well to wash about the alæ with sterile water and then have the patient blow his nose on a piece of sterile gauze and take the material for culture or smear from this. If the material is purulent and located at some ulcerating spot, it is best to use a speculum, and either touch the spot with a sterile swab or use a capillary bulb pipette with a slight bend at the end.

Normally, we find only white staphylococcus colonies and colonies of short-chain streptococci. The *M. tetragenus*, *B. xerosis*, and Hofmann's bacillus are also occasionally found.

In some cases of ozena we may find an organism of the Friedländer type in pure culture.

Biscuit-shaped diplococci, both Gram-negative and positive, are to be found either normally or in cases of coryza. *M. catarrhalis* has probably been frequently reported as the *Meningococcus*. Still, the *Meningococcus* has been found in the nasal secretions of patients with cerebrospinal meningitis originating most probably in the posterior pharynx. *B. Influenzæ* and the *Pneumococcus* have also been frequently found in cultures from the nasal secretions. The cause of the contagious type of coryza is a filterable virus.

Diphtheria involving the nasal cavity must always be kept in mind, and in quarantine investigations the examinations of the nasal secretions culturally should be a part of the routine.

The tubercle bacillus may be found in nasal ulcerations; it is, however, only present in exceedingly small numbers. On the other hand, one of the best diagnostic procedures in leprosy is to examine smears from nasal mucous membranes for the *B. lepræ*. In such ulcerations the bacilli are found in the greatest profusion. Rarely glanders may cause ulcerations.

B. proteus is frequently responsible for the production of foul odors in nasal discharges but does not seem to produce inflammatory conditions of the nasal mucosa. It simply decomposes the discharges. Various fungi have been reported from the nose, but in such a region the strictest conservatism in reporting should be observed.

Recently sporozoa have been reported in a case of nasal polyp. (*Rhinosporidium*).

So many degenerative changes in epithelial cells resemble protozoal forms that such findings require ample confirmation.

The larval form of *Linguatula rhinaria* is a rare parasite of the nasal cavities; it is not infrequent, however, in the nostrils of dogs.

Various fly larvæ are far more common, and the "screw-worm," the larva of the *Chrysomyia macellaria*, is common in certain parts of tropical America, and may by its burrowing effects cause fatal results.

The larvæ of *Sarcophaga* have in particular been found in the nasal cavities of children. Myriapods, while of very little importance elsewhere, have been reported more than 30 times from the nasal fossæ.

In a study of the bacteriology of otitis media, in 277 cases, Libman and Celler found streptococci present alone in 81%, *Streptococcus mucosus* in 10% and the *Pneumococcus* in 8%; *Staphylococcus*, *B. pyocyaneus* and *B. proteus* have also been found. Mixed infections are common.

Streptococci are the organisms which most often cause sinus thrombosis and brain abscess. The influenza bacillus has been reported as a cause of acute otitis media.

Nonvirulent diphtheroid bacilli are not infrequently obtained in cultures from ear discharges. Meningococci may cause otitis media.

Other organisms which have been isolated from middle-ear or mastoid discharges are *B. coli*, *M. catarrhalis*, *M. tetragenus* and Friedländer's bacillus.

B. typhosus may be found in middle-ear discharges of persons who have had an attack of typhoid fever.

The middle ear is normally free of bacteria, but in affections of the throat, as with streptococci, pneumococci, and diphtheria bacilli, these organisms may infect it by way of the Eustachian tube.

The moulds are of greater importance in affections of the external auditory canal than the bacteria. The cerumen seems to make a good culture medium so that various species of *Aspergillus*, *Mucor*, etc., develop and close the canal. These infections are often introduced by the patient's finger. Various mites and fly larvæ have been reported from the ear.

The "screw worm," the larva of *Chrysomyia macellaria*, is the most common cause of aural myiasis in tropical America. The fly deposits its eggs about aural and nasal cavities of those with offensive discharges. The larvæ develop and cause intense pain and giddiness. Larvæ of *Sarcophaga*, *Calliphora* and *Anthomyia* have also been reported from the external auditory meatus. The tympanic membrane may be perforated.

CHAPTER XXVI

EXAMINATION OF BUCCAL AND PHARYNGEAL MATERIAL

In a preparation made from material taken by a sterile swab from the region of the normal buccal and pharyngeal cavities and stained by Gram's method we are struck by the variety of organisms present. The fungi of thrush are best examined for in a preparation of membrane mounted in 10% caustic potash solution. *Monilia* may be found in sprue ulcerations about tongue or buccal mucosa. In examining for buccal amœbæ mount the purulent material from pyorrhœa in the

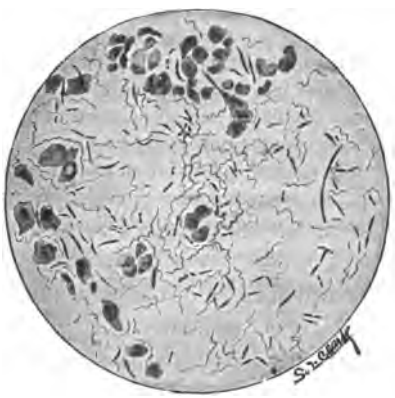


Fig. 131.—Vincent's angina. *Spirochæta vincenti.* (*Coplin.*)

patient's saliva. Smears from about carious teeth often show the fusiform bacillus and delicate spirillum of Vincent as well as cocci.

Gram-positive and Gram-negative staphylococci are normally present, as are also streptococci, pneumococci, leptothrix forms, and very probably yeasts and sarcinæ types with many Gram-negative bacilli. If pseudo-diphtheria organisms are present, we have these showing a Gram-positive reaction. If this material is smeared on agar plates and cultured at 37°C., we are struck by the fact that the colonies on the plates may be exclusively staphylococcal and streptococcal.

Of course diphtheroids as well as diphtheria organisms grow well on ordinary agar plates. For *Meningococcus* carriers a blood agar plate is advisable.

It is very difficult, if not impossible, to distinguish a *Pneumococcus* colony from a *Streptococcus* one on a plate culture. The presence or absence, however, of the *Pneumococcus* is distinctly shown in the Gram-stained smear, either by its lance-shaped morphology or the presence of a capsule. It has been my experience that smears from about 15% of normal individuals show capsulated pneumococci.

In diphtheria examinations we rely chiefly on the cultural findings on Löffler's serum. Where the process is streptococcal or due to the organisms associated with Vincent's angina, the immediate examination of a smear from the suspected spot or area gives greater diagnostic information. The *Streptococcus* being so abundant in cultures from normal throats, it is difficult to determine its significance in a culture; abundance of streptococci in a smear from an ulceration or bit of membrane, however, is of etiological import. Streptococcal sore throats are often very toxic and may be fatal—often milk borne. Use blood agar plates to differentiate hæmolyzing and "viridans" types of streptococci.

It would seem that carriers of *S. hæmolyticus* among the measles patients in service camps have been the chief factors in spreading this streptococcal infection to othes measles cases as a result of the coughing and sneezing incident to the disease.

Measles with its tendency to bronchial irritation seems to make conditions ideal for the development of a streptococcal bronchopneumonia in those harboring *S. hæmolyticus* in their throats.

By staining with Neisser's method it is possible to make an immediate diagnosis of diphtheria from a smear from a piece of membrane in about 25% of cases. It is well, however, to always culture such material. The toluidin blue stain of Ponder is the best stain for diphtheria.

Material from the throat is ordinarily best obtained with a sterile copper-wire cotton-pledget swab. The platinum loop usually bends too easily. A sterile forceps may be more convenient for obtaining particles of membrane. It is believed that ulcerative conditions of the throat, associated with the presence of the large fusiform bacillus and delicate spirillum, which make the picture of Vincent's angina, are more common than is usually so considered.

In Giemsa-stained smears from the dirty membrane covering the ulcerated area of Vincent's angina there are usually two types of the fusiform bacillus to be seen; one rather slender, pale blue with maroon dots at either end, the other rather thicker and of a uniform maroon staining. The spirilla are from 10 to 18 microns long and the fusiform bacilli from 5 to 7 microns.

As a rule, only cultures on Löffler serum are made and very rarely direct smears. If a smear were always made and stained by Gram's method (with a contrast stain of dilute carbol fuchsin) at the same time the culture was made, it is probable that much information of value would be obtained.

The *B. fusiformis* is an anaerobe which gives a fetid odor but culturally has no distinct characteristics. The spirillum has not been cul-

tivated. It has been thought that the bacillus and spirillum are different stages of the same organism.

At times aggregations of the fusiform bacillus give the appearance of branching so characteristic of diphtheria organisms. Being Gram-negative, however, the differentiation is easily made—the *B. diphtheriæ* being Gram-positive. Again the attenuated ends of the fusiform bacillus are diagnostic.

It is usually stated that the fusiform bacillus is nonmotile. By mounting material in saliva I have noted a sluggish, but distinct motility. The fusiform bacillus and spirillum are often associated with cocci and amœbæ in pus from dental caries or pyorrhœa and I mount such material in the patient's saliva to obtain motility in the amœbæ. The fusiform bacillus is not markedly Gram-negative.

The culturing of material from the nasopharyngeal region of contacts as well as patient is very important in outbreaks of cerebrospinal fever. Use a bent wire applicator with sterile cotton tip and pass it to the nasopharynx avoiding the uvula. Inoculate tubes or plates of blood agar immediately and keep them warm until placed in the incubator.

Direct smears are the procedure of choice in streptococcal and pneumococcal anginas as well as in Vincent's angina.

Unless very familiar with the morphology of *Treponema pallidum* and using dark field or Fontana's staining procedure, we should be very conservative in reporting such an organism from suspected syphilitic ulcerations of the throat.

We now know that we have treponemata in the buccal cavity similar to *T. pallidum* so that even with the dark field illumination I would base a diagnosis on the clinical and Wassermann reactions rather than morphologically.

The thrush fungus (*Endomyces albicans*) may be easily demonstrated in a Gram-stained specimen as violet mycelial structures.

Yeasts due to food particles are not infrequently observed in smears and cultures from the mouth.

Actinomycosis may develop about a carious tooth and the finding of the ray fungus in the granules from the pus may give the diagnosis.

Amœbæ and flagellates have been reported from the mouth. For a time *Endamœba buccalis* was considered the exciting cause of pyorrhœa alveolaris, this organism being frequently obtained from scrapings about affected teeth or in the contents of root abscesses. Emetin was the accepted treatment. At present we consider the rôle of these amœbæ as of doubtful importance. Also in the remarkable disease "halzoun," flukes have been found to be the cause of the asphyxia.

In the tropics, round worms (*Ascaris*) may be vomited up and, lodging in the pharynx, may have to be extracted.

During the campaign of Napoleon in Egypt many cases of leech involvement of the nasal and buccal cavities were noted. The parasite was the *Limnatis nilotica* which gained access to the upper pharynx through drinking water from springs and pools. Many such cases continue to be reported from the Mediterranean basin.

CHAPTER XXVII

EXAMINATION OF SPUTUM

FREQUENTLY the material submitted for examination as sputum is simply buccal or pharyngeal secretion, or more probably secretion from the nasopharynx, which has been secured by hawking. It should always be insisted upon that the sputum be raised by a true pulmonary coughing act, and not expelled with the hacking cough so frequently associated with an elongated uvula. When there is an effort to deceive, some information may be obtained from the watery, stringy, mucoid character of the buccopharyngeal material and also from the presence of mosaic-like groups of flat epithelial cells (often packed with bacteria). The pulmonary secretion is either frothy mucus or mucopurulent material, and if the cells are alveolar they greatly resemble the plasma cells. At times these cells may contain blood pigment granules (heart-disease cells).

In the microscopic examination a small, cheesy particle, the size of a pin head, should be selected. This should be flattened out in a thin layer between the slide and cover-glass and should be examined for elastic tissue, heart-disease cells, eggs of animal parasites, amœbæ, and fungi. *Echinococcus* hooklets, Curschman spirals besprinkled with Charcot-Leyden crystals, and hæmatoidin and fatty acid crystals may also be observed.

Curschman spirals indicate bronchial as against cardiac or uremic asthma. Charcot-Leyden crystals have no special significance, except in certain tropical diseases when these crystals often are present in paragonomiasis sputum and in the pus of amœbic liver abscesses discharging by way of the lungs.

It may facilitate the examination of the sputum for elastic tissue and actinomycosis and other fungi to add 10% sodium hydrate to the preparation. Elastic fibres are highly refractile, wavy and tend to show branching. Unless they show an alveolar arrangement we cannot be sure they do not come from elastic fibers of our food. The yellow actinomycosis granules can be recognized by the eye and under the low power of the microscope show as nodular finely granular bodies. The threads and clubs show up with higher powers.

T. B. Staining.—To make smears for staining, the sputum should be poured on a flat surface, preferably a Petri dish, and a bit of mucopurulent material selected with forceps. A dark back-ground facilitates picking out the particle. A toothpick is well adapted to smearing out such material on a slide. After using the toothpick it can be burned. When dry, the smear is best fixed by pouring a few drops of alcohol on the slide, allowing this to run over the surface, and then, after dashing off the excess of alcohol, to ignite that remaining on the film in the flame and allow to burn out.

A mark with a grease pencil about ½ inch from the end, gives a convenient surface to hold with the forceps and also prevents the stain subsequently used from running over the entire surface. A piece of glass tubing about 12 inches long bent into a narrow V shape makes a very satisfactory rest for the slide in staining and is convenient for the steaming of staining solution over the flame.

Sputum should as a routine measure be stained by the Ziehl-Neelson method and by Gram's method.

In examining for tubercle bacilli it may be necessary to employ some method for concentrating the bacterial content of the sputum prior to making the smear. A very satisfactory method is that of Mühlhäuser-Czaplewski. Shake up the sputum with four to eight times its volume of ¼% solution of sodium hydrate in a stoppered bottle. When the mixture has become a smooth, mucilaginous-looking fluid, add a few drops of phenolphthalein solution and bring the pink mixture to a boil.

Then add drop by drop a 2% solution of acetic acid, stirring constantly, until the pink color is just discharged. If the least excess of acid is added over that just sufficient to cause the pink color to disappear, mucin will be precipitated. Now pour this mixture into a centrifuge tube and smear the sediment on a slide and stain for tubercle bacilli.

Antiformin.—Tubercle bacilli usually occur nested in clumps of sputum. Therefore, when few in number it is only by chance that they may be found. Concentration methods aim to dissolve these clumps of sputum and collect, free from mucus, whatever bacilli may be present.

There are many concentration methods for sputum. One of these has been given above. Uhlenhuth's method has some advantages over others in the solvent used: 1. It breaks up the sputum very rapidly; 2. it immediately dissolves all organisms except acid-fast ones; 3. applied in not too concentrated form and for not too long a time, tubercle bacilli are not killed, so that by washing the sediment carefully by several dilutions and centrifugings we have in the sediment viable tubercle bacilli which we may attempt to cultivate upon Dorsett's or other suitable media with the reasonable hope that contaminations will not choke them out or prematurely kill the inoculated guinea-pig; 4. it has less effect upon the staining properties of tubercle bacilli than any other material used in concentration methods. Petroff's method is probably better.

To make this solvent (antiformin) take double the quantity of chlorinated lime and sodium carbonate required by the U. S. Pharma-

copœia and prepare according to U. S. P. directions. To the finished liquor sodæ chlorinatæ (Labarraque's solution) add 7½% of sodium hydrate.

The Liquor sodæ chlorinatæ of the Br. P. is slightly stronger and some English authorities recommend a mixture of equal parts of this Labarraque's solution and 15% sodium hydrate solution. As a rule 1 part of antiformin to 5 parts of sputum is sufficient. Very tenacious sputum may require 1 part to 4 parts of sputum. If more antiformin is used the specific gravity is too much increased and the bacilli are damaged. The fluidification is hastened at incubator temperature.

To 5 parts of sputum add 1 part of antiformin, shake well and place in incubator for one hour. To 10 c.c. of the homogeneous mixture add 1.5 c.c. of a solution made up of 1 part chloroform and 9 parts alcohol. Shake violently and centrifuge for fifteen minutes. Mix the sediment with egg albumin, smear out and stain.

When it is desired to culture the tubercle bacilli mix 20 c.c. of sputum with 65 c.c. sterile water and add 15 c.c. antiformin. Stir the mixture with a glass rod. After thirty minutes to two hours we should have a homogeneous mixture. Centrifuge for fifteen minutes or longer, wash the sediment twice with sterile salt solution and smear out the well-washed sediment over serum or glycerine egg slants. The tubes should be covered with black paper and the plugs paraffined. It must be remembered that for culturing tubercle bacilli we must protect the growth from sunlight as this will kill the organism. If fluid culture media are inoculated the transferred material should be deposited on the surface. Should the particle sink growth will not occur.

Sputum smears stained by some Romanowsky method or by the hæmatoxylin-eosin stain are best adapted for the study of various cells, and in particular of the eosinophile cells so characteristic of bronchial asthma. In sputum from cancer of the lungs the large vacuolated cells may be found.

In making a smear of the tenacious rusty sputum of lobar pneumonia we note the value of a Gram stain in bringing out the capsulated pneumococci. Of course a special capsule stain may give slightly better results.

In bronchopneumonia the sputum may be somewhat streaked with blood but it rarely shows the blood admixture of the sputum of lobar pneumonia.

It is a mucopurulent sputum, smears from which are filled with pus cells, and cultures most often show streptococci as cause. In the measles bronchopneumonias which have given so high a death rate in the camps the cause is almost always *S. hæmolyticus*. At times the influenza bacillus is associated with it. These bronchopneumonias have given a high percentage of empyema complications. They have also given a mortality approximating 50 per cent.

When examining the sputum of the bronchopneumonia of influenza the formol fuchsin gives the best results. The influenza bacilli are found in little masses, frequently grouped about small collections of *M. tetragenus*. The cocci stain a rich purplish-red, while the small influenza bacilli take on a light pink color.

A greenish yellow, nummular sputum, often profuse, is frequently noted in influenza.

T. B. sputum showing a mixed infection with streptococci or pneumococci or with the influenza bacillus makes for a bad prognosis. *M. tetragenus*, which often is present when cavities exist, does not seem to be so unfavorable prognostically.

Red cells show up well in specimens stained by the Romanowsky method; if rouleaux formation is marked, it may indicate pulmonary infarction.

In culturing sputum a mucopurulent mass should be washed in sterile water and should then be dropped into a tube of sterile bouillon. With a sterile swab it should be emulsified and successive streaks made along the surface of an agar, blood agar or glycerine agar plate. In obtaining cultures from influenza sputum, first smear the material thoroughly over a blood-serum slant; then inoculate, by thorough smearing over the surface of successive blood-streaked agar slants, the material on the surface of the blood-serum slant. The platinum loop should be transferred from one slant to another without recharging. The influenza bacillus seems to grow better if the blood-streaked agar slants are prepared just before inoculating with the sputum. All that is necessary is to sterilize an ear, puncture and take up the exuding blood with a large loop and smear over the agar slant. Cultures for tubercle bacilli are impracticable except with antiformin or by Petroff's method. This latter is most satisfactory. A guinea-pig should be inoculated.

The blood-stained watery sputum of plague pneumonia should be cultured on plates of plain agar and 3% salt agar at the same time. An ordinary smear stained with carbol thionin, however, practically makes a diagnosis. Be sure to inoculate a guinea-pig cutaneously.

Pneumococci, *M. catarrhalis*, and Friedländer's bacillus in sputum are best demonstrated by Gram's method of staining.

The distinct capsule staining of the pneumococci in a Gram preparation of sputum from a suspected case of pneumonia is of value in diagnosis.

The finding of the ray fungus (*D. bovis*) in sputum gives the diagnosis of actinomycosis. *Streptothrix* infections of lungs have been confused with tuberculosis.

Moulds, especially aspergilli, may be found in sputum. Species of *Mucor*, *Cryptococcus*, and *Endomyces* have also been reported.

Amœbæ from liver abscess rupturing into the lung may be found. Very important pulmonary infections are those with *Paragonimus westermanii*. This is recognized by the presence of operculated eggs in the sputum.

A fluke, *F. gigantea*, was once found in sputum.

Hydatid cysts, either of the lung or of the liver, rupturing into the lung, may be recognized by the presence of echinococcus hooklets. The material is bile-stained if from the liver. Dutcher has reported filarial embryos from sputum.

Strongylus apri has been reported once from the lungs and embryos might be found in the sputum.

The test for ALBUMEN IN THE SPUTUM is of value in the diagnosis of pulmonary tuberculosis.

About 10 c.c. of fresh sputum as free as possible from saliva is mixed with an equal quantity of water and 2 c.c. of a 3% solution of acetic acid to remove mucin. After filtering the filtrate is tested for albumin. The test is obtained also in pneumonia and pleurisy with effusion.

CHAPTER XXVIII

THE URINE

MATERIAL for staining is best obtained by centrifuging the urine, then pouring off the supernatant urine, then draining the mouth of the centrifuge tube against a piece of filter-paper so that we have only the pus sediment to finally remove with a capillary bulb pipette or toothpick stuck in a urine sediment pipette and make smears.

I always take up the material with the centrifuge tube in a slanting position following the draining off of the supernatant urine to avoid urine admixture in the smear as such makes staining less satisfactory. The Gram staining is most satisfactory, counterstaining with Bismarck brown.

The addition of a loopful of egg albumen or blood serum to about twice that amount of urinary sediment gives better results. (See under Staining Methods.)

In pathological urine there is enough albumin to fix the smear.

The smear may be stained after fixing by heat with Gram's stain, T. B. stain, or hæmatoxylin and eosin. The latter is the best for the staining of epithelial cells and animal parasites; the Gram method for bacteria.

It is frequently difficult to distinguish the spores of moulds from red blood-cells except by measurement and staining reactions. Spores of moulds rarely exceed five microns.

Of the greatest value is the finding of phagocytized bacteria in the pus cells of the Gram-stained smear. These indicate the causative organism which show beautifully in the beginning of pyelitis infections. To examine for epithelial cells I make a vaseline streak across a slide about ¾ inch from the center. A drop of the sediment is deposited on the slide which may then be examined unstained with the ⅔ inch objective and then a drop of Gram's iodine solution is added. One edge of a square cover-glass is rubbed into the vaseline line and allowed to drop on the fluid preparation. Currents are avoided and the cells stain up beautifully.

It is difficult to determine the presence of blood in urine in higher dilution than 1 to 300 with the spectroscope. The ordinary occult blood test will show it in much higher dilution.

To secure urine for bacteriological examination catheterization is rarely necessary in men—in the case of women it is the proper method.

Authorities generally insist upon a catheterized specimen in all cases when the urine is to be cultured. As a matter of fact when there is a bacterial infection the specific organism is in such predominant numbers that it is easily distinguished from a possible contaminator. Of course should one culture the urine in a tube of

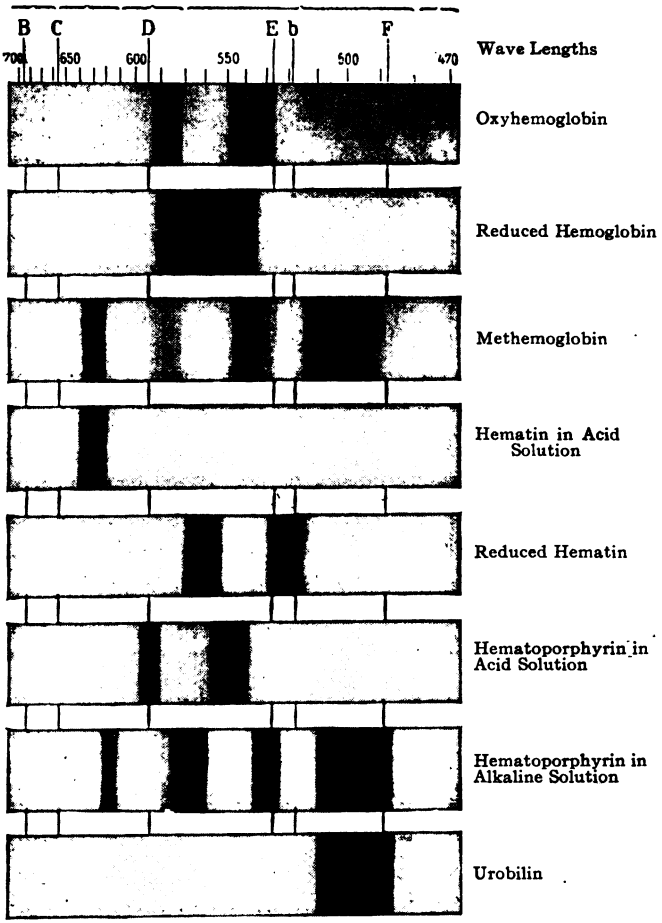


FIG. 132.—The Most Important Clinical Spectra. (*Monographic Medicine.* D. Appleton & Co., New York.)

bouillon, before plating out, a contamination might overgrow the causative organism, but one should always plate directly from urine which has just been passed. The smear stained by Gram's also checks up, particularly if certain bacteria are found phagocytized in pus cells. The man who follows the clinical side as well as the laboratory one is rarely confused by an occasional contaminating organism on a plate

made from urine or blood. Of course the problem is more difficult with urine, but when culturing of urine is a routine procedure the worker soon knows the organisms likely to be encountered in urine of women as well as that of men. As a matter of fact I rarely find colonies on plates made from the urine of normal men, the only precautions taken being those noted below. I now use blood agar plates as routine plating media.

The glans penis and meatus should be thoroughly washed with soap and water, after which dilute alcohol (70%) should be used. The greater part of the urine first passed should be rejected and only the last portion passed should be caught in a sterile receptacle. A drop of this urine may be either streaked over the surface of an agar, blood agar, or a lactose litmus agar plate, or so treated after being first diluted in a tube of sterile bouillon.

The lactose litmus agar medium is very useful in distinguishing typhoid or paratyphoid colonies (blue) from colon, and *Streptococcus* or *Staphylococcus* colonies (pink). The urine may be added to tubes of melted agar and then poured.

The most satisfactory procedure is to deposit one drop on a poured plate and five drops on a second plate. The surface is smeared over with a bent glass rod first smearing out the single drop and then going to the second plate without a second sterilization. Neutral glycerine agar or blood agar is desirable for such organisms as pneumococci or streptococci and, for the *Gonococcus*, Thalman's medium smeared over with a few drops of human serum or Vedder's starch agar.

Cystitis from a colon infection gives an acid urine; that caused by *Proteus vulgaris* an alkaline urine.

The old designation *B. termo* so often employed in connection with the bacteriology of the urine in older works applied to the proteus group and *M. ureæ* to ordinary staphylococci.

The bacillus of typhoid and the micrococcus of Malta fever are also found in the urine. This elimination in urine of bacilli by typhoid carriers is of great importance in the spread of the disease.

While the smegma bacillus in urine may be differentiated from the tubercle bacillus by the former losing its red color, by prolonged decolorization with acid alcohol, yet it is chiefly by the subcutaneous inoculation of the guinea-pig that we should diagnose genito-urinary tuberculosis. Inject the sediment after centrifuging.

The method recommended by Gasis which depends on the alkali fast properties of the T. B. has not given me satisfactory results.

Smegma bacilli are not disintegrated by antiformin as are other bacilli than the tubercle one, so that treatment of urinary sediments with antiformin for finding tubercle bacilli does not differentiate those of smegma.

Gonococci are reported from Gram-stained smears.

To culture *Gonococcus* material the transfer to culture media should be made almost immediately after obtaining the material from the patient. *M. catarrhalis* is a rare finding.

Staphylococcus and *Streptococcus* infections about the throat as well as such infections in heart or joint may show the presence of the causative organisms in the urine. At times bacterial infections of the kidney may give symptoms of renal stone.

As it is much easier to culture urine than blood a bacteriological examination of the urine may give us the desired information and the organism for the autogenous vaccine. Salt mouth bottles with cotton plugs, when sterilized, make cheap and satisfactory containers. The urine should be plated out as soon as possible after its passage. As a rule when organisms are present in the urine they are in such numbers that the question of contamination rarely arises.

Yeasts and moulds frequently contaminate urine, especially diabetic urine, after it has been passed. Amœbæ and flagellates (*Trichomonas vaginalis* in females) may be found in urine.

Eggs of *Schistosoma hæmatobium* (bilharziosis) are important diagnostic findings; these are terminal-spined. Those of rectal bilharziosis are, as a rule, lateral-spined.

In chylous urine the filarial embryos may be found. This examination is facilitated by centrifugalization.

The eggs of the *E. gigas* may be recognized in urinary sediment by their pitted appearance.

The vinegar eel may be found in the urine of females who have used vaginal douches of vinegar.

Echinococcus hooklets, scolices, or laminated membrane have been found in the urine.

The larval dibothriocephalid, *Sparganum mansoni*, has been reported three times in urine (urethra).

Oxyuris from the vagina may be found in urine.

Various mites may be found in urinary sediment as the result of lack of care in the washing of the receptacle and are entirely accidental.

Unless having the characteristics of the itch mite and in a person showing scabies lesions about the genital organs the diagnosis of the mite as *A. scabiei* should not be made.

Crystals of biliverdin may be found in the urinary sediment in marked jaundice. They somewhat resemble crystals of tyrosin but are brownish in color while those of tyrosin are black. Furthermore, it is excessively rare to find crystals of leucin and tyrosin in the urinary sediments, and in such diseases as acute yellow atrophy of the liver, the urine should be concentrated to one-tenth its volume and the residue treated with alcohol. The tyrosin crystalline sheaves and the leucin striated globules crystallize out from the alcohol.

URINARY SEDIMENTS

Turbidity of the urine is most often due either to bacterial contamination, amorphous urates (sedimentum lateritium) or phosphates.

Urates go into solution upon heating and phosphates upon the addition of a few drops of acetic acid.

In turbidity due to bacteria contaminating the urine subsequent to its passage it is best to call for another sample.

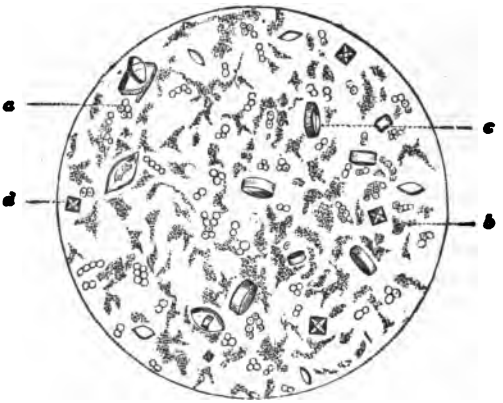


FIG. 133.—Deposit in acid fermentation. *a*, Fungus; *b*, amorphous sodium urate; *c*, uric acid; *d*, calcium oxalate.

To preserve urinary sediments formalin is the best for casts and epithelial cells while for general use one may employ a piece of camphor or the addition of one volume of saturated borax solution to four volumes of urine.

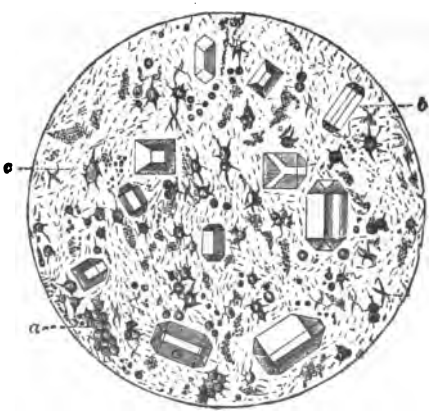


FIG. 134.—Deposit in ammoniacal fermentation. *a*, Acid ammonium urate; *f*, ammonium magnesium phosphate; *c*, bacteria.

Chloroform does not answer for sediments as it does for urine to be examined chemically. To take up a sediment insert a pipette to the bottom of the tube with the opposite opening closed by a finger, then tease the sediment into the pipette opening in the centrifuge tube, by manipulating the fingers.

In a urine of acid reaction we may find the following unorganized sediment:

I. Amorphous sodium or potassium urates. Usually yellowish red. Heat and alkali bring about solution.

II. Uric acid. Whetstone crystals of yellowish-red color. Soluble in alkalis but not by heat. Abundant sediment of uric acid crystals may be due to too great concentration or too great acidity of the urine rather than to the so-called uric acid diathesis.

III. Calcium oxalate. Octahedral crystals or dumb-bell shapes which are highly refractile. Often due to diet (asparagus, tomatoes, spinach, rhubarb, etc.).

IV. Cystin occurs in six-sided crystals which are soluble in ammonia.

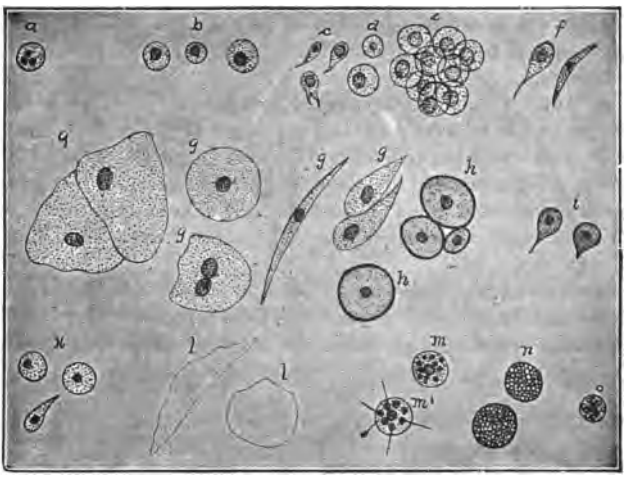


FIG. 135.—Epithelium from different areas of the urinary tract. *a*, Leukocyte (for comparison); *b*, renal cells; *c*, superficial pelvic cells; *d*, deep pelvic cells; *e*, cells from calices; *f*, cells from ureter; *g*, *g*, *g*, *g*, *g*, squamous epithelium from the bladder; *h*, *h*, neck-of-bladder cells; *i*, epithelium from prostatic urethra; *k*, urethral cells; *l*, *l*, scaly epithelium; *m*, *m'*, cells from seminal passages; *n*, compound granule cells; *o*, fatty renal cell. (*Ogden.*)

In a urine of alkaline reaction we may expect:

I. Triple phosphates (NH_4 $MgPO_4$). Usually in coffin-lid or fern-like form. Easily soluble in acetic acid.

II. Calcium phosphate and calcium carbonate which effervesce on the addition of acid.

III. Ammonium urate. These show as the thorn-apple structures.

The presence of ammonium urate, particularly if with triple phosphates, denotes bacterial decomposition within the genito-urinary tract provided the urine is just passed. Pus cells derived from the site of inflammation should be present also. While certain bacteria might possibly bring on chemical changes without giving rise to inflammation yet such a possibility is so rare as to be negligible. In the pres-

ence of amorphous phosphates one should always think of exogenous sources as vegetable diet or withdrawal of proteid food before thinking of disordered metabolism.

Organized Sediment.—An occasional leukocyte may be found in the urine of healthy people. Any abundance of leukocytes indicates inflammation of genito-urinary tract. Some workers count the pus cells in urine by the same technic used for the leukocyte count of the blood. A urine having 100,000 pus cells per c mm. will give as a result about 0.1% albumin.

Leukocytes are found in abundance at times in the urine of women without pathological significance. Red blood cells usually show as pale doubly ringed bodies. They appear in inflammations, particularly stone or schistosome infection, also with neoplasms or in chronic passive congestion. They may be found in conditions where toxins are being eliminated through the kidneys, as in tuberculosis. The menstrual period of women must be kept in mind in the examination of urine sediments.

Epithelial Cells.—For morphology of cells from different locations see illustration. It is almost impossible to state positively the origin in the genito-urinary tract of certain cells. Very trustworthy evidence however is finding of epithelial cells on casts or the so-called compound granule cells (fatty degenerated renal epithelium). Sheets of more or less small round or caudate epithelial cells are rather significant of pyelitis. Vaginal epithelium resembles that gotten from scraping the buccal mucosa.

Bladder epithelium resembles vaginal but is of smaller size, and ureteral is like that of the pelvis of the kidney but smaller. Cells from the region of the prostate are very refractile with a distinct nucleus and are oval rather than round.

Casts.—Of casts we have (1) hyaline, narrow and homogenous. They do not prove nephritis. (2) Epithelial casts. Usually indicative of nephritis but very slight inflammatory processes can cause them. (3) Blood casts. (4) Granular casts. If coarse granules rather significant of chronic nephritis. Finely granular casts do not seem to have any more significance than hyaline ones. As a matter of fact under dark ground illumination hyaline casts show a granular structure. (5) Waxy casts are highly refractile, show fissuring of margins and were formerly considered of serious prognostic import (chronic nephritis), but the present view is that they are only ordinary casts which have been retained in the renal tubules for a long time. Even amyloid kidney does not produce any distinctive cast.

Cylindroids are drawn-out bodies showing tapering ends, irregularity of diameter and longitudinal striations.

It will be found that a ⅔-inch objective gives almost all the information required as to casts. It is quicker and gives more positive information.

Mounting a sediment in Gram's solution or tinging it with the merest trace of neutral red is of much assistance.

Starches and Fibers.—In examining urinary sediments it is important to be familiar with the various textile fibers and starch grains which are so frequently present, the fibers coming from the clothing and the starch grains from dusting powders. Wool fiber fragments show bark or scale-like imbrications and are round. Cotton fibers are flattened and twisted, while linen ones show a striated flattened fiber with frayed segments as of a cane stalk. Silk shows a glass-like tube with mashed in ends.

Corn and rice grains are the most common of the starch grains and their nature is immediately disclosed by their blue color when mounted in iodine.

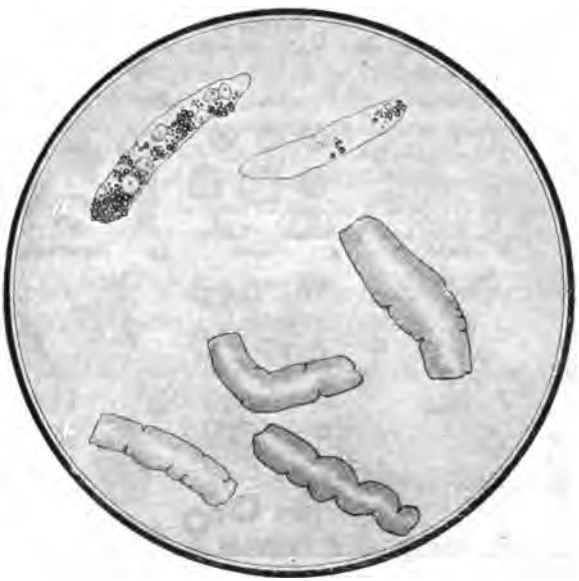


FIG. 136.—Fatty and waxy casts. *a*, Fatty casts; *b*, waxy casts. (*Greene.*)

Hæmaturia.—By this we mean the presence of red blood cells in the urine, and the condition is often recognizable only by microscopic or occult blood examinations. It is better to separate this condition from hæmoglobinuria. Origin may be renal, cystic, urethral, ureteral and secondary to disease or traumatism. It may also result from affections of structures adjacent to the genito-urinary apparatus, especially ulcerations of the large intestines (amœbic), or from disease of the female generative apparatus as uterus, vagina or tubes.

In certain general diseases, as smallpox, purpura, and leukæmia one may expect hæmaturia. In the tropics it is a finding in yellow fever (asthenic stage) and in plague as well as in bilharziasis and hæmatochyluria of filariasis.

Hæmorrhage from the kidneys may arise from malignant growths, especially hypernephroma, or from benign ones, as papilloma of the pelvis.

Hæmaturia occurs not only in acute nephritis but in some chronic cases. Chronic passive congestion is associated with red cells in the urine and renal infarctions from endocarditis may also bring it about.

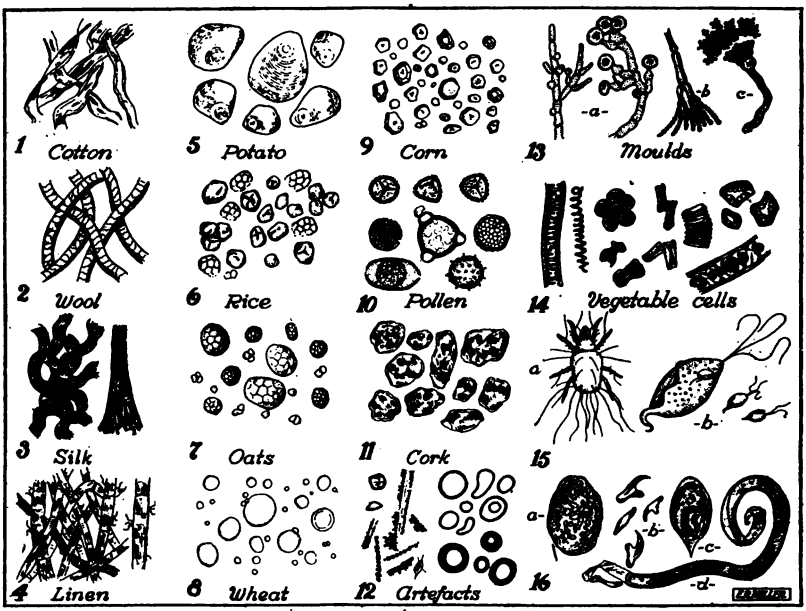


FIG. 137.—Fibers, starch granules, etc., which may be found in urine sediment. No. 12 gives appearance under microscope of scratches on old used glass slides. No. 15(*a*), *Tyroglyphus longior*, a mite. No. 15(*b*), *Trichomonas vaginalis*. No. 16 (*a*), Egg of *Eustrongylus;* (*b*), *Echinococcus hooklets;* (*c*), *Schistosoma* egg; and (*d*), *Filaria bancrofti* embryo.

Stone in the pelvis of the kidney is an important cause. In the bladder we have as chief causes (1) tumors, malignant or innocent, and (2) calculus. In tumors the bleeding is not markedly controlled by rest as is that from stone.

In the urethra gonorrhœal inflammation, especially when near the neck of the bladder, and traumatism may be associated with hæmaturia.

Hæmoglobinuria.—The two diseases one always thinks of in connection with hæmoglobinuria are blackwater fever and paroxysmal hæmoglobinuria. It is discussed under tests for transfusion.

Bacteriuria.—Bacterial infections of the genito-urinary tract are associated with more or less *pyuria*. Kidney infections are now recognized as most often from the blood stream rather than from extension from portions of the tract lower down.

The most important hæmatogenous bacterial infections of the kidney are, in order of frequency, colon bacillus, staphylococci, streptococci, gonococcus, proteus, typhoid and paratyphoid. Renal tuberculosis is generally hæmatogenous rather than an ascending infection. Very important is the differentiation of the pyuria of cystitis and pyelitis. Of course bladder irritation will follow pyelitis.

The use of two sedimentation glasses will differentiate pus from the urethra from that from bladder and pelvis of kidney. If the urine in the first glass alone is turbid it shows urethral pus. With cloudiness of the contents of the second glass the problem is more difficult because it is almost impossible to differentiate a bladder involvement from a renal one by microscopic examination. Cystoscopy is necessary. Of course, some authorities attach importance to the character of the pelvis epithelium, others to the acid urine of pyelitis and the alkaline one of cystitis. Again it is often noted that the albumin content of the urine of pyelitis is far greater than that from cystitis. This is true for pyelonephritis but does not hold for simple pyelitis.

Pyelitis, usually a colon infection, must always be thought of in vague disorders of children and pregnant women.

Renal stone and renal tuberculosis often give similar symptoms but the X-ray and animal inoculation test should differentiate.

Determination of Efficiency of Renal Functioning

At present we are paying great attention to laboratory tests which give us an idea of the activity of nitrogen metabolism and efficiency of the renal functions. Probably the most reliable single test is the phenolsulphonephthalein or "red" test. This is given in the appendix. For alveolar CO^2 content see Acidosis.

Determination of the ammonia output in the urine is also of value in conditions of acidosis. In acidosis connected with diabetes we expect a great increase in the urinary ammonia to neutralize acetone bodies as is also true of degenerations involving the parenchymatous liver cells, when the urea function is interfered with. In the acidosis of chronic nephritis, however, we may have a deficiency in the ammonia output in the urine. Simple tests for this deter mination will be found in the appendix.

Great attention is being given to the nonprotein nitrogen of the blood as well as to blood urea. See appendix.

The study of nitrogen metabolism is best undertaken with a patient on a known nitrogen value diet and the most accurate determination is along the lines of the Ambard index of urea excretion.

Ambard Index.—McLean has worked out an index of urea excretion based on the Ambard variables of (1) concentration of urea in the blood. (2) Concentration of urea in the urine. (3) Rate of urinary excretion and (4) weight of the individual. McLean's formula is as follows:

$$\frac{D\sqrt{C} \times 8.96}{Wt \times Ur^2} = \text{Index of urea excretion.}$$

D = Grams urea excreted in twenty-four hours.
C = Grams urea per liter urine.
Ur = Grams urea per liter blood.
Wt = Body weight, individual in kilograms.

One hundred is accepted as a typical normal finding, and findings down to 80 as within normal limits. In deficiency of renal function the index falls below 50 and in cases of marked deficiency may fall below 10, and in terminal stages the index may approximate 1. Such cases practically show an absence or only a trace of phenolsulphonephthalein excretion.

For determination of urea excretion the patient drinks 200 c.c. water and one-half hour later empties his bladder. Commencing at this we wait seventy-two minutes (one-twentieth of twenty-four hours) and then collect the urine for urea determination.

The blood for urea determination should be taken midway in the period of collection of the urine—thirty-six minutes after the bladder is voided.

Salt Retention.—As a test for salt retention Schlayer advises giving 10 grams of NaCl to a patient having a salt equilibrium as the result of a known diet continued over several days. This added salt should be eliminated in the urine within twenty-four hours or certainly by forty-eight hours. Monakow in a similar way gives 20 grams of urea at one dose and this added urea should be eliminated in twenty-four to forty-eight hours to show normal nitrogenous output.

A case of nephritis may show a good urea elimination but salt retention.

Diminished capacity to eliminate salt is a rather constant finding in all types of chronic nephritis.

Frothingham considers elimination of 8.5 grams of the 10 grams administered as normal, above 4.5 grams as slight retention and below 4.5 grams as marked retention.

Lactose Excretion.—Intravenous injection of 20 grams lactose in 20 c.c. distilled water. The solution should be pasteurized at 80°C. for

three hours on three successive days. The urine is collected at one- to two-hour intervals and tested with Nylander's test for sugar until you get negative reaction. Normally all lactose is excreted in four or five hours.

Nephritic Test Meal of Mosenthal.—A very valuable method of estimating renal function is the response to the nephritic test meal of Mosenthal. This test meal contains approximately 13.4 gm. of nitrogen, 8.5 gm. of salt, inclusive of the salt in the capsules, 1760 c.c. of fluid and considerable purin material.

The test diet is the following:

Breakfast, 8 A. M.:

Boiled oatmeal, 100 gm.; sugar, 1 to 2 teaspoonfuls; milk, 30 c.c.
Two slices bread (30 gm. each); butter, 20 gm.
Coffee, 160 c.c.; sugar, 1 teaspoonful; milk, 40 c.c.
Milk, 200 c.c.
Water, 200 c.c.

Dinner, 12 Noon:

Meat soup, 180 c.c.
Beefsteak, 100 gm.
Potato (baked, mashed, or boiled) 130 gm.
Green vegetables as desired.
Two slices bread (30 gm. each); butter, 20 gm.
Tea, 180 c.c.; sugar, 1 teaspoonful; milk, 20 c.c.
Water, 250 c.c.
Pudding (tapioca or rice), 110 gm.

Supper, 5 P. M.:

Two eggs cooked any style.
Two slices bread (30 gm. each); butter, 20 gm.
Tea, 180 c.c.; sugar, 1 teaspoonful; milk, 20 c.c.
Fruit (stewed or fresh), 1 portion.
Water, 300 c.c.

8 A. M. No fluid or food is to be given during the night or until 8 o'clock the next morning (after voiding), when the regular diet is resumed.

Patient is to empty bladder at 8 A. M., and the end of each period, as indicated below. The specimens are collected for the following periods in properly labeled bottles: 8 A. M. to 10 A. M.; 10 A. M. to 12 N.; 12 N. to 2 P. M.; 2 P. M. to 4 P. M.; 4 P. M. to 6 P. M.; 6 P. M. to 8 P. M.; 8 P. M. to 8 A. M. If specimens are not examined at once, a preservative such as thymol or chloroform should be added, or specimen should be kept in ice box.

The salt is furnished in weighed amounts in three capsules, each capsule containing 2.3 grams of salt. One capsule for each meal.

The diet contains substances which act as diuretics and the mode of excretory response indicates normal or pathological renal function. As showing a normal response to the diet Mosenthal gives the following table:

Time of day	Urine, c.c.	Sp. gr.	Sodium chloride, gm.	Nitrogen, gm.
8–10	153	1.016	2.02	1.26
10–12	156	1.019	1.95	1.15
12–2	194	1.012	1.24	1.14
2–4	260	1.014	2.00	1.46
4–6	114	1.020	1.13	1.08
6–8	238	1.010	1.02	1.23
Total day	1115	.....	9.36	7.32
Night, 8–8	375	1.020	2.36	4.61
Total 24 hours	1490	.....	11.72	11.93
Intake	1760	.....	8.5	13.4
Balance	+270	.....	−3.22	+1.47

To quote Mosenthal:

"The test is a qualitative one of the mode of urinary function as measured by the specific gravity, salt, nitrogen and water excretion in two-hour periods during the day, and for a twelve-hour period at night. The normal individual yields specimens with specific gravity figures which vary ten points or more from the highest to the lowest, a night urine high in specific gravity, 1.018 or more, high in its percentage of nitrogen—above 1 per cent.—and small in amount —400 c.c. or less. The quantities of water, salt and nitrogen excreted approximate the intake.

When kidney function becomes involved, the first signs are usually demonstrated in the night urine, the quantity becomes increased; the specific gravity and the nitrogen concentration are lowered. One or all of these changes from the normal may occur. In severe cases of chronic nephritis, an advanced degree of functional inadequacy of the kidney is indicated by a markedly fixed and low specific gravity, a diminished output of both salt and nitrogen, a tendency to total polyuria and a night urine showing an increased volume, low specific gravity and low concentration of nitrogen."

	Amount and character of urine	Sp. gr.	Albumin	Sediment	Etiology	Special features
Acute nephritis	Diminished.....	High 1025 to 1030	High 0.5% and more.	Large amount. Hyaline granular epithelial and blood casts. Renal epithelium. Red and white blood-cells.	Infectious diseases. Chilling. Poisons.	Sudden onset. Œdema often marked, especially of face. Mild or even severe uræmic symptoms. Pulse tension increased but heart not hypertrophied.
Ch. parenchym. nephritis.	Normal or diminished.	Moderately high 1020 to 1025.	Large amount. 0.5 to 2%.	Abundant. All kinds of casts including fatty and waxy. Red blood-cells. Much fatty epithelium.	Following acute attack. Alcohol, syphilis, malaria. T. B. suppuration.	Marked œdema. Uræmia common. Hypertrophied left ventricle. Blood pressure increased.
Ch. interstitial nephritis.	Greatly increased (2000 to 4000 c.c.). Bright color.	Low 1005 to 1010.	Trace; rarely exceeding 0.1%.	Very slight. Very few hyaline and granular casts. Red and white blood-cells.	Heredity, gout, syphilis, alcohol, lead, arteriosclerosis.	No œdema until later. High blood pressure (200 to 250). Cardiac hypertrophy; often uræmia and albuminuric retinitis.
Passive congestion.	Diminished; high color.	High 1025 to 1030.	Small amount.	Sedimentum lateritium; occasional hyaline casts. Red and white blood-cells. Renal epithelium exceptional.	Ch. heart and lung disease.	No uræmia. Symptoms attributable to heart.
Pyelitis.....	Normal, slight turbidity. Often hæmaturia.	Normal.	Slight.	Abundance of pus cells. Caudate epithelium often in clusters. At times red blood-cells.	Bacterial infection ascending or descending. Stone.	Reaction of urine acid. No tenesmus.
Cystitis.....	Normal but very turbid.	Normal.	Slight.	Very great abundance of pus cells. Much mucus and bacteria.	Bacterial infection.	Reaction of urine alkaline or very faintly acid. Tenesmus.

CHAPTER XXIX

THE FÆCES

IT is advisable to examine a stool macroscopically before taking up the microscopical examination. The mucus shreds or casts of the bowel in mucous colitis or membranous enteritis may give the diagnosis of obscure abdominal pain. Pus in stools may often be noted without the aid of the microscope.

The normal stool is sausage shaped and soft. Neither the special form of scybalous masses called sheep pellets nor the pencil-like nor the tape-like excrement prove the existence of stricture of the intestinal lumen although suggestive of such a condition. The mucus of bacillary dysentery is opaque and grayish from the great number of pus and phagocytic cells. It is well to remember that Charcot-Leyden crystals, which are practically always absent from bacillary dysentery stools, are not infrequent findings in the amœbæ containing stools; of course, these crystals appear in other intestinal parasite infections.

In obstruction of the common bile duct we have acholic, whitish, foul-smelling stools. If the putty color be due to bacterial change exposure to the air will restore the brownish tinge.

Sprue stools are white-wash to putty colored, pultaceous, and filled with air bubbles. The amount is excessive.

Fatty stools are best examined microscopically.

As so many solid masses resemble gallstones it is well to dissolve the suspected mass in hot alcohol and examine for cholesterin crystals upon evaporation of the alcohol.

If the fæcal examination is to be made for the diagnosis of amœbæ, in a case where the characteristic mucus stools are not present, or to verify the existence of flagellates, it is best to give a dose of salts early in the morning and examine the liquid stools which follow such treatment. This treatment is satisfactory for examination for intestinal parasites or ova.

A very practical way of obtaining amœbæ is to pass a rectal tube or a piece of drainage tube with fenestrations into the bowel, and amœbæ may be found in the mucus filling the perforations in the tube. Walker advises against the use of salts in examinations for amœbæ.

Test Diet.—If the purpose of the examination is to determine the digestive power of the alimentary tract for proteids, carbohydrates, or fats, it is best to use a test diet, as that of Schmidt and Strasburger.

Prior to using this test diet, one should familiarize himself with the macroscopic and microscopic appearances resulting from such a diet in a normal person; information is then at hand to judge of variations from the normal. The examination of the fæces of persons, on ordinary and specifically undetermined articles of diet, is very unsatisfactory when the state of digestion of muscle fibers and the question of fat digestion are at issue.

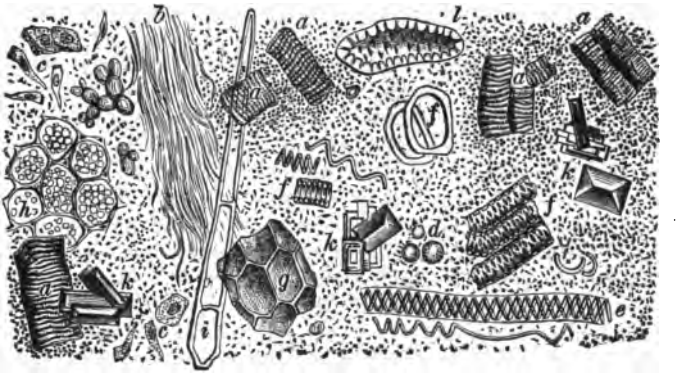


FIG 138.—Microscopical constituents of fæces. (*v. Jaksch.*) *a*, Muscle fibers; *b*, connective tissue; *c*, epithelium; *d*, leukocytes; *e*, spiral cells; *f*, *g*, *h*, *i*, various vegetable cells; *k*, "triple phosphate" crystals; *l*, woody vegetable cells; the whole interspersed with innumerable microörganisms of various kinds.

In examining the fæces of the normal person and likewise with the patient, wait until the second or third day so that the fæces of previous diets may have passed out. A charcoal powder taken before commencing the diet serves as an indicator.

Diet: breakfast, 7 A. M., bowl of oatmeal gruel (40 grams oatmeal, 10 grams butter, 200 c.c. milk, 300 c.c. water). Also one very soft-boiled egg (one minute) and 50 grams zwieback. In the forenoon, 500 c.c. of milk.

For dinner, 2 o'clock, chopped beef broiled very rare (125 grams with 20 grams butter poured over it.) Also a potato puree (200 grams mashed potato, 50 grams milk, 10 grams butter). Also ½ liter of milk and 50 grams zwieback.

For supper, 7 o'clock, the same articles as for breakfast.

This detailed diet may be varied to suit circumstances as regards interchanging meals. Furthermore, the milk may be taken in the form of tea or cocoa or cooked with the other food. Even a small amount of wine may be permitted. The diet taken, however, should absolutely conform to the following requirements: 1. the taking of ¼ pound chopped beef, a portion of which should be half raw; 2. the milk taken should amount to about a quart; 3. about 4 ounces of bread or toast and from 4 to 8 ounces of potato puree should be eaten daily.

The detailed diet contains about 110 grams albumin, 105 grams fat and 200 grams carbohydrates with a fuel value of 2247 calories.

The stool is best collected in quart fruit jars and examined as soon after evacuation as possible. The wooden spatula-like tongue depressors are well adapted to handling the specimen.

Having familiarized one's self with the degree of digestion of muscle, starch, and fat in a normal person, we are in a position to judge of the state of assimilation in a patient.

The first part of the test is the macroscopical one. For this grind up a fæcal mass of ½ to 1 inch diameter in a mortar, gradually adding water until it has the consistence of a broth. About ½ c.c. of this emulsion should now be squeezed out between two slides and studied against a dark surface and then when held up to the light. The normal stool gives a rather uniform brownish homogeneous layer. Connective-tissue remnants (indicative of gastric derangement) show as whitish fibers. Undigested muscle tissue remnants as reddish-brown splotches. Fat particles as whitish-yellow clumps. Potato remnants appear like sago grains and mash out easily like mucus. Mucus is best noted in the fæcal mass before making the emulsion. *In the microscopical test of this emulsion:* We judge of muscle digestion by the intactness of the striations. If a muscle remnant is only a homogeneous yellowish particle, it shows satisfactory digestion. If it is rectangular, with well-defined cross striations, it shows poor digestion for meat (Azotorrhœa). A loopful of fæces should be smeared into a drop of Gram's solution for starch-digestion determination. Normally there should be no blue staining starch granules.

Soaps are gnarled bodies everted like the pinna of an ear, while soap crystals are comparatively coarse and do not melt on application of gentle heat as do the more delicate fatty acid crystals. Neutral fat is in round or irregular globules. The best stain for fat is Sudan III (saturated solution of Sudan III in equal parts of 70% alcohol and acetone).

Mix up the fæces with dilute alcohol (50 to 70%) and then add a drop of the above solution and apply a cover-glass quickly. The fat globules show as orange or golden yellow bodies.

By rubbing up a small portion of the fæces in 36% acetic acid, applying a cover-glass and heating over a flame until the preparation shows bubbles, we convert the soaps and other fat combinations into free fatty acids which show as more or less numerous highly refractile bodies showing a crystalline structure as the preparation cools. By practice one learns the amount of such globules to expect with different fat contents in stools.

Steatorrhœa, or the presence of fat in abnormal quantities in the fæces, is shown by the pale, bulky, greasy stools as well as in the microscopical examination.

Average for normals in 1 gram dried fæces:

Total fat,	225 mg.	(22.5%)
Total fatty acid,	86 mg.	(37.9% of all fat)
Total soap,	74.7 mg.	(33.4% of all fat)
Total neutral fat,	64.4 mg.	(28.5% of all fat)

In normal cases the only fat elements recognizable are yellow calcium or colorless soaps. In sprue from 25 to 30% of the fat ingested appears in the stool while the stool of pellagra shows only about 5% which is the normal figure.

As quantity of fat increases (as say 500 to 600 mg.) droplets of neutral fat appear with or without increase in number of soap masses. Also needles and splinters of fatty acid and soaps appear. Much connective-tissue débris shows defect in gastric digestion, as only the stomach digests connective tissue.

A test for activity of fermentation should be made by using a Schmidt apparatus.

A distinct evolution of gas in twelve hours shows starch digestion defect. Such fæces are acid. A delayed production of gas (after twenty-four hours) shows albumin decomposition. Such fæces show an alkaline reaction. The apparatus is shown in Fig. 9. Into a stocky salt mouth bottle we put aproximately 5 grams of fæces which have been rubbed up into an emulsion with water and fill the bottle with water. The remaining portion of the apparatus consists of a test-tube or a graduated cylinder fitted with a doubly perforated rubber stopper. One U-shaped glass tube passing through this stopper connects with a second test-tube. This tube serves as a receptacle for any water which may come over from the water-filled tube or graduated cylinder and has an opening punched out of the bottom of the test-tube. The other opening in the twice perforated cork admits a straight tube which connects with a large rubber stopper which fits into the bottle for the fæces. To prepare, fill the graduated cylinder, then push in the doubly perforated cork which is connected with the side receiving tube and the large rubber cork. This latter is then pushed down to fit tightly into the bottle filled full with the fæces emulsion.

In addition to the fæces examination we should check the results from the test diet with indican and nitrogen partition determinations of the twenty-four-hour urine specimen—the ratio of ammonia nitrogen to total nitrogen indicating the functional power of liver and the indican the question of stasis in lower part of small intestine.

The most satisfactory test for bile in the fæces is to emulsify a small particle of fæces in a saturated aqueous solution of bichloride of mercury, preferably with a wooden toothpick, on a concave glass slide. After one or more hours hydrobilirubin-containing fæces show a salmon pink color and bilirubin ones a green color. One should familiarize himself with these reactions in normal cases. The examination of fæces for bile is less certain than duodenal fluid examination.

Amœbæ in stools.—In examining a liquid stool after salts, it is well to color the drop of fæces, which is to be covered with the cover-glass, with a small loopful of ½% solution of neutral red. If diluting fluid is used, it should be salt solution, and not water. The neutral red tinges the granules of the endoplasm of amœbæ and flagellates a very striking rose pink color, thus differentiating them from vegetable cells or body cells.

Whether examining the thin fæces or the mucus particle, it is well to reserve report on amœbæ or flagellates until motion is observed. Encysted protozoa are difficult to diagnose. An experienced examiner easily recognizes the four or eight nucleated cysts when the material is mounted in Gram's iodine solution.

Bacterial Smears.—When a smear preparation is desired, we may smear out a fragment of mucus and stain by Romanowsky's or Gram's method. The character of the bacteria present appears to be of diagnostic value—especially in the case of infants and young children. Beautiful preparations may be made by mixing the fæces with water, then centrifuging for one minute. This throws down vegetable débris and crystals. Now decant the supernatant fluid, which holds the bacteria in suspension, and add an equal amount of alcohol. Again centrifuge, decant, and smear out and examine the bacterial sediment.

Simply taking a small mass of fæces and emulsifying it with a wooden toothpick on a concave slide in 70% alcohol—then, after the sediment settles, taking up a loopful with platinum loop from the surface and smearing out, gives a very satisfactory smear. Gram's method, with dilute carbol fuchsin counterstaining, gives the best picture.

The Boas-Oppler bacillus may be found in the stools in this way. Normally, a Gram-stained stool shows a great preponderance of Gram-negative bacilli and such a finding in a measure excludes cancer of the stomach. Organisms which are Gram-positive as well as the Boas-Oppler bacillus are, 1. Lactic acid bacilli—these show Gram-negative areas in the slender bacilli. 2. A type of bacillus similar in size to the colon bacillus but Gram-positive and noncultivable (found in acid stools). 3. Bacilli of the *B. subtilis* type.

It is very important to examine the fæces for T. B. With children a diagnosis of tuberculosis may be made in this way when the sputum cannot be obtained, the pulmonary secretion being swallowed. The preparation on the concave slide as described above should be stained for T. B. Ulcerations in intestinal T. B. may show very numerous bacilli.

To culture for typhoid, dysentery, cholera, or other bacteria, take up the material in a tube of sterile bouillon and smear it out with a swab over a lactose litmus agar plate or an Endo or Conradi-Drigalski plate. Before streaking the plates they should be very dry on the surface. This can be best done by pouring into a plate with a circular piece of filter-paper in the lid and placing in the incubator for one-half hour to dry. The filter-paper absorbs the moisture. Then inoculate the surface of the plate with the fæcal material. Selective cholera plating media are strongly alkaline.

In summer complaints of infants and children the organisms concerned are as a rule related to various dysentery strains of bacilli. Kendall in 293 stool examinations found the gas bacillus (*B. ærog. capsul.*) in 22 cases. The gas bacillus produces intestinal disorders which are not benefited by lactose but by buttermilk (lactic acid bac-

teria). For diagnosis, a loopful of the fæces is emulsified in a tube of sterile milk or litmus milk. The emulsion is heated to 80°C. and held at this temperature for twenty minutes. After incubation for eighteen to twenty-four hours, preferably anaerobically, we get (1) a shreddy disruption of the casein, (2) the smell of rancid butter and (3) fully 80% of the casein is dissolved. Smears show short thick Gram-positive rods with slightly rounded ends. *B. subtilis* is sometimes found but does not give a rancid odor nor the strong disruption of the clot.

Pancreatic Functioning.—It was until recently thought that Cammidge's reaction (urine) when associated with azotorrhœa and steatorrhœa made for a diagnosis of chronic pancreatitis. At present very little importance is attached to the Cammidge reaction.

Loss of weight, anæmia, diarrhœa and pains in the upper abdomen are important indications of pancreatic trouble. As chronic pancreatitis is often associated with cholelithiasis jaundice is frequently present. Glycosuria is not often present. While functional tests are important they do not make for a sure diagnosis. At present we examine the duodenal fluid for presence of pancreatic ferments.

Müller's method for pancreatic functioning determination is to give a calomel purge two hours after a meal. A little of the liquid stool is smeared on the surface of blood-serum and the tube incubated at 60°C. (paraffin oven). If the surface is smooth, no trypsin was present; if dotted with spots of digestion liquefaction, it shows that the pancreatic secretion is present.

In Schmidt's nucleus test small cubes of beef are hardened in absolute alcohol and then tied up in tiny silk bags. These are recovered from the fæces and sectioned. Complete preservation of nuclei indicates a total absence of pancreatic functioning provided the passage of the tissue be not too rapid as by diarrhœa.

In the microscopic examination, epithelial cells are generally more or less disintegrated. In the mucus of bacillary dysenteryc stools, however, large intact phagocytic cells are frequent, which may be mistaken for encysted amœbæ.

Triple phosphate crystals are frequently observed in fæces as may also be crystals of various calcium salts. Charcot-Leyden crystals are rather indicative of helminthiases.

Various flagellates, and in particular *Lamblia*, may be responsible for diarrhœal conditions which may cause rather serious symptoms.

Balantidium coli has been reported several times as the cause of dysenteric conditions. Coccidiadea are found in the fæces.

Isospora was not an infrequent finding in the stools of the soldiers at Gallipoli.

Gall-stones.—Gall-stones are usually recognized by their facetted appearance. The stool should be examined for two weeks following an attack of hepatic colic and the fæces should be rubbed up in water and

passed through a sieve. A concentric arrangement of layers is usually noted on fracturing a gall-stone. For identification dry and pulverize the stone and treat with alcohol and ether. This dissolves the cholesterin and upon evaporation the rhombic crystals separate out and may be recognized microscopically. The residue may be extracted with cold dilute KOH solution. This extracts bilirubin which may be recognized by Gmelin's test. Pseudo-gall-stones are usually masses of fats, soaps or vegetable material.

At times enteroliths are mistaken for gall-stone. These are shells of inorganic salts covering inspissated masses of fæces or seeds, etc.

It is in the fæces we examine either for the parasites or for their ova in connection with practically all the flukes, except the lung fluke and the bladder fluke; for intestinal tæniases and for practically all the round worms, except the filarial ones.

Bass has recommended that fæces which have been made fluid be centrifuged and the supernatant fluid containing vegetable débris poured off. The sediment contains hookworm eggs. Then pour on sediment a calcium chloride solution of sp. gr. 1050. Again centrifuge and decant. Next add calcium chloride solution of a sp. gr. of 1250 and centrifuge. This brings to the surface the hookworm eggs which may be pipetted off. As a rule, the finding of hookworm eggs is very easy without such a technic. The eggs of *Trichostrongylus* greatly resemble those of hookworm but are larger, 73 to 91μ long. In perfectly fresh fæces *Strongyloides* are present as worm-like embryos while hookworm gives only two to four segment eggs. See Barber's method under hookworms.

In the tropics, the examination of the fæces vastly exceeds in value that of urine and is possibly more important than blood examinations.

The larvæ of various insects may at times be detected in the stools, as well as certain acarines (cheese mites, etc.).

The test for occult blood is indicated in helminthiases as well as in the conditions for which it is usually tested.

CHAPTER XXX

BLOOD CULTURES AND BLOOD PARASITES

CLINICALLY, the most important examinations of the blood for parasites is for the presence of various bacterial infections and for certain blood protozoa and also filarial embryos.

The modern method of culturing blood, especially for the detection of typhoid or paratyphoid bacilli, is by the use of the bile media of Conradi. Test-tubes are filled with 7 to 10 c.c. of 1% peptone ox bile, or ox bile alone, and the medium is sterilized in the autoclave. It is good practice to place the syringe in a plugged test-tube containing salt solution, with the needle unscrewed. After autoclaving, the sterile syringe can be taken to the bedside in the test-tube. Using a wide test-tube, a forceps can be sterilized at the same time and used to attach the needle to the barrel of the syringe.

By using a piece of glass tubing into which the needle is inserted we may sterilize the syringe easily in the test-tube. The glass tubing prevents the steel needle from coming in contact with the glass of the test-tube and so prevents cracking the test-tube.

The skin should be scrubbed gently with green-soap solution and water for about three minutes. The skin of the area to be punctured should then be sterilized by the gentle application of Harrington's solution (not scrubbed) for one-half minute, and should then be washed with sterile water. It appears to be safe to simply scrub the area with 70% alcohol for one or two minutes. Applications of pure carbolic acid on a gauze wad for a few seconds followed by neutralization with 70% alcohol gives satisfactory sterilization. *The present method of sterilizing skin* for taking blood or inoculating vaccines is simply to smear the site of entrance for the needle rather heavily with tincture of iodine. A tourniquet is now applied to distend the vein, and the needle, beveled side up, is inserted in the direction of the venous flow. Withdrawing 5 to 10 c.c. of blood, we loosen the tourniquet (otherwise the blood may flow from the puncture) then withdraw the needle, and force out about ½ c.c. into the first bile tube, about 1 c.c. into the second, and 2 or 3 c.c. into the third. It is well to reserve some of the blood for Widal tests.

The bile tubes are now incubated for ten to twelve hours and then transfers are made to bouillon tubes. These bouillon tubes can be used in six to eight hours for testing the organism against known typhoid or paratyphoid sera. Test-tubes containing 10 c.c. of ordinary bouillon with 1% of sodium citrate are as satisfactory as bile media.

Some prefer a 2% sodium glycocholate in bouillon while others use a 2% solution of ammonium oxalate in bouillon for blood cultures.

Some prefer to streak plates of lactose litmus agar with material from the bile tubes instead of inoculating the bouillon tubes. Contamination with staphylococci or the presence of staphylococci, streptococci, or plague bacilli in septicæmic conditions show easily accessible colonies.

Schotmuller adds 1 to 3 c.c. of blood to liquefied agar at 45°C., and after mixing pours into plates. The standard method formerly was to add the blood to an excess of bouillon (1 to 5 c.c. of blood to 100 c.c. or more of bouillon).

The method of culturing blood we now follow in our laboratory is the following:

A stout hypodermic needle is attached to about 6 inches of rubber tubing which in turn is pushed over a downward bent glass tube which passes through a doubly perforated rubber stopper. A second glass tube, which also passes through the stopper, is bent upward to be attached to a second piece of rubber tubing for use in suction by the mouth. We keep on hand sterilized pieces of glass tubing packed with a little cotton to prevent the possibility of any saliva getting into the system. These are mouth pieces and are inserted after the tubes and stopper are sterilized. The glass tubes project about ½ inch below the undersurface of the rubber stopper and above are about 2½ inches including the bent arm. This system of tubing and stopper is readily sterilized by boiling in a pan or instrument sterilizer. As a receptacle for the blood we employ Erlenmeyer flasks of 100 c.c. capacity, containing 10 to 25 c.c. of salt solution with 1 or 2% of sodium citrate, for prevention of coagulation. These citrated salt solution flasks are plugged with cotton, sterilized and kept on hand ready for immediate use, so that we only have to sterilize the stopper and tubing by boiling and flame the neck of the flask when removing the cotton plug to insert the stopper of the system. By suction we can take any amount of blood desired. I usually count the drops of blood as they fall into the citrated salt solution allowing 16 drops to the cubic centimeter. In this way we may take from 10 to 25 c.c. of blood at the bedside and then later on in the laboratory, when it is convenient, inoculate various media from the flask. For plates add 2 or 3 c.c. of this citrated blood to 6 or 8 c.c. of melted agar at 45°C. The blood mixture can also be added to various sugar bouillons for fermentation reaction. Finally we place the receiving flask in the incubator and culture it as well as the other media. Some organisms do not develop in this citrated blood, possibly on account of the blood concentration. This can be avoided by adding 50 to 75 c.c. of bouillon to the citrated blood remaining in the flask.

Of course in inoculating the various plating or sugar tubes from the flask there is some liability to contamination. This may be avoided by removing with a sterile pipette 10 to 20 c.c. from the flask containing the citrated blood to carry out the inoculations instead of pouring out directly from the flask. See Fig. 9.

A very useful procedure in the isolation of streptococci, pneumococci, plague and anthrax bacilli is to inject 1 to 2 c.c. of blood into suitable animals. When infecting mice use only about 0.2 c.c. subcutaneously at root of tail or, more certain of results, the injection of

about 1 c.c. of the blood intraperitoneally. Streptococci, even from virulent human infections, are uncertain in their action on animals so that the failure to produce septicæmia in the mouse does not necessarily indicate that the organism is of slight virulence.

By using the bile media, we can take the blood from the ear in typhoid cases, if preferred. Then if chance staphylococcic contamination occurs, such colonies are readily differentiated from typhoid ones by the pink color on lactose litmus agar. For culturing blood in septicæmic conditions, the blood should always be drawn from the vein and cultured either by mixing 1 to 2 c.c. with melted agar and then pouring plates or by transferring to bouillon in excess (at least ten times as much bouillon as blood) and after eighteen to twenty-four hours' incubation plating out. For *Streptococcus* and *Pneumococcus* blood agar plates are to be preferred, the *Pneumococcus* giving green colonies with only a suggestion of hæmolysis while the *Streptococcus* gives an opaque colony with a distinct hæmolytic zone surrounding it. We rarely culture blood anaerobically as the important pathogenic anaerobes (tetanus, gas gangrene and malignant œdema) do not tend to invade the blood stream during life. Recently a case has been reported where the gas bacillus was isolated from the blood of a soldier with gas gangrene. There is an anaerobic streptococcus *S. putridus* which grows anaerobically on blood agar giving porcelain white colonies without hæmolysin. The cultures have a putrid odor. Not pathogenic for animals.

Bacteriæmia.—Warren and Herrick have recently published a very important study of 134 cases of bacteriæmia. Bacteriæmia signifies the mere presence of bacteria in the blood without reference to symptoms, while sepsis denotes conditions due to invasion of the blood stream by bacteria or their toxins with marked systemic reaction.

Of 25 cases with endocarditis 22 died and 3 were unimproved. Of 55 cases of sepsis 39 died and 10 recovered. In postpartum infections 10 died and 1 recovered. In osteomyelitis 5 died and 4 recovered. In otitis media 2 died and 4 recovered.

Thirty-one cases were due to *Streptococcus hæmolyticus* of which 21 died.

Forty cases were due to *S. viridans* and 25 died. One case from *S. mucosus* died.

Of 39 cases with *Staphylococcus aureus* 22 died and in 3 cases of *S. albus* 2 died.

Of 10 cases of *Pneumococcus* bacteriæmia 6 died. Six of *B. coli* infection gave 4 deaths; two of *B. influenzæ* 2 deaths; three of anaerobic streptococci, 2 deaths and two of *B. mallei*, 2 deaths.

Seven cases of mixed infections gave 6 deaths.

The ordinary leukocyte and differential count procedures were of very little value in prognosis.

The average white count in fatal cases of *S. hæmolyticus* infection was 18,347 with 81% of polymorphonuclears while in cases recovering it was 18,742 and 85%, respectively. Fatal *S. viridans* infections gave an average white count of 15,976 with polymorphonuclear percentage of 78, while nonfatal cases gave 17,222 and 75%, respectively.

Of 25 cases treated with vaccines, 81% died; while of 47 under surgical treatment, 50% died and with 50 treated palliatively, 50% died.

Blood Cultures.—Typhoid cultures are best obtained in the first week of the disease, after that time the Widal is the test of preference.

If a paratyphoid serum is not at hand for testing, it may suffice to inoculate a glucose bouillon tube or a Russell lactose glucose litmus slant; gas production indicates paratyphoid. This test should be applied when a very motile organism, cultured from the blood, does not show agglutination with a known typhoid serum. Anthrax and glanders should be considered in blood cultures.

In Malta fever it must be remembered that colonies do not show themselves for several days. Addition of blood to melted agar is a good procedure.

Blood for culturing typhoid or the paratyphoids may be taken with a Wright's tube from the ear or finger. Dipping the hand in hot water assists the flow of blood. The supernatant serum after centrifugalization should be pipetted off with a sterile pipette and reserved for agglutination tests while the clot is dropped into a bile tube. (Clot culture).

Rosenberger was the first to insist upon the importance of examination of blood for T. B. Brem considered that many cases of finding of acid-fast bacilli were not of T. B. The Kurashigi-Schnitter method for tubercle bacilli in blood is to take about 1 c.c. blood and put in a centrifuge tube containing 5 c.c. of 3% acetic acid. After the red cells are thoroughly laked centrifuge, pipette off supernatant fluid and dissolve the sediment in 5 c.c. antiformin. When dissolved add 5 c.c. absolute alcohol and centrifugalize for twenty minutes. Smear out the sediment and stain.

The examination of the blood for the parasites of malaria, filariases, kala-azar and spirillum fevers has been discussed under their respective headings.

With trypanosomes from human trypanosomiasis, smears from gland juice or cerebrospinal fluid seem more satisfactory to examine than blood smears unless the blood is taken in 5 to 10 c.c. quantities and centrifuged in sodium citrate salt solution.

The latest method in the diagnosis of trichinosis is to take 5 to 10 c.c. of blood from a vein at the time of the migration of the embryos to the muscles (ten to twenty days). This is forced out into a centrifuge tube containing 3% acetic acid, and the sediment examined for trichina larvæ.

CHAPTER XXXI

THE STOMACH AND DUODENAL CONTENTS

FROM a microscopical standpoint there is comparatively little that is of value in the examination of the gastric contents; there is nothing very specific about the findings.

A test meal is not a necessity as in the chemical examination, but either vomitus or material withdrawn with a stomach-tube two or more hours after an ordinary meal suffice.

The most satisfactory specimen is one taken before the giving of the test meal.

The washings from the stomach are allowed to stand until the sediment has fallen to the bottom and an examination of this is made.

The microscopical diagnostic points in connection with distinguishing cancer of the stomach from nonmalignant dilatation are: 1. Fragments of cancer tissue. These are very rarely found and are most difficult to diagnose. 2. The presence of flagellates in the early stages of cancer (the so-called anacid stage preceding the development of lactic acid). As flagellates prefer an alkaline medium, they disappear after the acidity due to lactic acid comes on. 3. The presence of the Boas-Oppler bacillus. There are probably several organisms so designated. They are lactic acid producers and are characterized by being very large bacilli ($7 \times 1\mu$) and arranged in long chains which stretch across the field of the microscope. They are Gram-positive and do not form spores. They can be cultivated on media rich in milk or blood and are aerobic. They should only be reported when present in great abundance and in long chains. Heinemann thinks it probable that the Boas-Oppler bacillus, *Leptothrix buccalis*, and *B. bifidus* may be identical with *B. bulgaricus* (see under Milk).

4. The absence of sarcinæ and yeasts. The presence of these sarcinæ and fungi in vomitus is indicative of a simple dilatation.

In the diagnosis of cancer, other than the finding of tumor, gastric stasis, etc., much importance is attached to the absence of free HCl. This is best determined by the Gluzinski test. In the Mayo clinic total acidity of gastric contents averaged 63 (5/6 free HCl) in ulcer, while cancer averaged only 31 (1/3 free HCl). Lactic acid was found in 43% and blood, occult or otherwise, in 73% of cancer cases.

In determining the pepsin activity of the gastric juice we usually employ the Mett tube.

In chronic gastritis the picture of mucus entangling large numbers of cells is characteristic. It must be remembered, however, that strands of mucus entangling polymorphonuclear cells may be found in normal gastric contents. The pus cells which are free are the ones of significance. An occasional cylindrical gastric epithelial cell may be found. The recognition of carcinoma cells, especially those showing mitoses, is of value only when done by one who has made a special study of the findings.

In examining the sediment from the filter-paper after filtering off the stomach contents always use a dilute Gram solution (about 1 to 4) for mounting the sediment. Muscle fibers, yeast cells, red blood-cells, and epithelial cells are stained a golden yellow. Starch granules are stained blue while fats are unstained and show as globules of varying sizes.

The Duodenal Fluid.—This is best obtained with the Jutte modification of the Einhorn duodenal tube. There is little difficulty in the passage of the tube when the stomach is not dilated. One can aspirate from 5 to 20 c.c. in about five minutes, the amount varying with the individual case. The normal fluid is clear, bile stained, seromucous and contains few cells and living bacteria. Dead bacteria may be found in considerable numbers.

Of the greatest importance is the chemical examination of duodenal fluid for pancreatic ferments. It is well recognized that the examination of the fæces for such ferments is absolutely unreliable while with duodenal fluid it is most satisfactory. The normal ferment value of duodenal fluid is best indicated by the tryptic power, this being very constant. The lipase findings are very variable.

Normally 10 c.c. of 0.1% casein solution is digested in twenty-four hours by duodenal fluid in dilutions of 1 to 3000 to 1 to 10,000 or even higher.

Turbidity of the fluid is thought to be suggestive of cholelithiasis. Bile-stained pus cells are also of significance in cholecystitis. Hence examine sediment unstained before adding Gram's iodine solution. The presence of bile in the duodenal fluid is a far more certain index of the patency of the bile duct than the test of fæces for bile as bile may be absent in a fæces test when present in duodenal fluid. The color of the fluid is about as satisfactory an indication of the presence of bile as that given by the various tests for bile. Einhorn states that with turbid bile cholecystitis with gall-stones is almost always present if with fasting condition. Gall-stones without cholecystitis may give a clear fluid. Liver conditions, as neoplasms or high grade cirrhosis may give rise to turbid duodenal fluid.

McNeil attaches importance to the Wolff-Junghan's test of the duodenal fluid which he states gives turbidity up to the third or fourth tube, but never in the fifth or sixth one, normally. An increase would indicate some pancreatic, biliary or duodenal inflammation, provided we checked up the test on the gastric contents.

In duodenitis we have stringy mucus and rather considerable numbers of living bacteria.

Typhoid carriers are best recognized by culturing the duodenal contents. MacNeal, in culturing the organisms from the duodenal contents of 26 cases, found that few living bacteria were present although dead ones might be present in considerable numbers. The living organisms are as a rule Gram-positive cocci.

B. lactis aerogenes was found rather constantly by Gessner.

In connection with the possible significance of bacteria in the duodenal contents it is interesting to note the findings of Kelly in a bacteriological study of 413 cases of operations on the biliary tract by Deaver. About one-half the cases showed sterile bile but in the acute cases living bacteria were almost constantly present. *B. coli* was found in 30%, *B. typhosus* in 7%, *Staphylococcus pyogenes* in 2.9%, *Streptococcus* in 0.2%. Other organisms were more rarely found and 55% were sterile.

In recently studying the bacteria of the duodenal contents and subsequently the bile of a case of cholelithiasis with cholecystitis the only organism found was *B. coli.* This organism was markedly hæmolytic on blood agar plates.

For the carrying out of the various tests see appendix.

CHAPTER XXXII

EXAMINATION OF PUS

Pus may be collected for examination either 1. with a platinum loop, 2. with a sterile swab, 3. with a bacteriological pipette or 4. with a hypodermic syringe.

It is always well to make a smear and stain it by Gram's method at the same time that cultures are made. The Gram stain gives information as to the abundance of organisms in the pus and as to the probable findings in the culture. Pneumococci and streptococci are differentiated from the staphylococci in this way without the necessity of more or less extended cultural methods.

Smears from material examined for gonococci may show Gram-negative diplococci which, however, do not generally have the morphology of the *Gonococcus* They are furthermore extracellular.

The *M. catarrhalis* has been reported from urethral smears though very rarely. Diphtheroid organisms are not uncommon. Gram-positive cocci are rather common in smears from discharges of chronic gonorrhœa.

When autogenous vaccines are to be made, the isolation of the exciting organism is necessary. This is best done by streaking the pus, taken up with a sterile swab and emulsified in a tube of bouillon, over the surface of an agar plate. Practically as convenient and providing a more nutritious medium is to smear the material on a loop or swab over the surface of a blood-serum slant, then to inoculate a second tube from the first without recharging the loop or swab, and so on until three or four tubes are inoculated. Isolated colonies should be obtained in a third or fourth tube.

In examining blood-serum slants inoculated with purulent material, always examine the water of condensation for streptococci.

A bacteriological pipette is very useful when pus is to be sent to a laboratory; the tip can be sealed in a flame and the cotton plug at the other end insures the noncontamination of the contents. The material may be drawn up either with the mouth or with a rubber bulb.

The hypodermic syringe is very useful in puncturing buboes, etc., especially in plague. A small pledget of cotton on a toothpick dipped into pure carbolic acid and touched to a spot over the bubo, which after about thirty seconds is soaked with alcohol, makes a sterile anæsthetic spot at which to introduce the needle of the syringe. It must be re-

membered that when plague buboes begin to soften, the plague bacilli may be replaced by ordinary pus organisms. The pus from wounds infected with anaerobes is usually very foul. The most important anaerobe in the discharge from gas gangrene wounds is *B. perfringens*.

In a study of the aerobic bacterial flora of war wounds Lawrence found that more than 80% of the discharges from such wounds showed streptococci, which especially flourished in deep pockets, staphylococci replacing them in shallow wounds. Gram-negative bacilli were present in 95% of smears. Of these *B. coli* was present in 50% of cases. *B. cloacæ*, *B. rhinoscleromatis* and *B. alkaligenes* were isolated in from 10 to 20% of wounds. As the suppuration continued *B. pyocyaneus* became frequent. The aerobic spore bearers, especially *B. subtilis*, *B. mesentericus*, *B. vulgatus*, *B. megatherium*, *etc.*, were observed in about 20% of wounds but disappeared promptly under treatment. The combination of aerobes and anaerobes in a wound make conditions more favorable for the anaerobes. Wounds contaminated with *B. fusiformis* do badly. In carrying out the Carrel treatment, daily smears are made of the discharge from the most markedly suppurating part of the wound. These are stained by Gram's method. The wound is not closed until the bacteria are so reduced in number that only one or two organisms can be found in each oil-immersion objective microscopic field.

The pus from the necrotic center of climatic bubo is sterile.

It is remarkable how frequently we get pure cultures from abscess material. In purulent material from abdominal abscesses we are apt to obtain mixed cultures, especially the colon bacillus and *B. pyocyaneus*, in addition to ordinary pus organisms.

When it is a question between streptococci and pneumococci, it is well to inoculate a mouse; the capsulated pneumococci at the autopsy make the diagnosis.

Animal inoculation is also necessary in plague and glanders, and possibly anthrax. When tetanus is suspected, it should be examined for as described under Tetanus. Tuberculosis should also be identified by inoculating a guinea-pig, as well as by acid-fast staining and culture, if there is any doubt as to the nature of the material.

The black or yellow granules of madura foot, as well as those of actinomycosis, should be examined as recommended in the section on fungi.

Amœbæ, coccidia, and larval echinococci may be found in purulent material, as may also various other animal parasites, as fly larvæ, sarcopsyllæ, etc.

The pus from an amœbic abscess of the liver is as a rule sterile when cultured.

The examination at the time of operation or exploration frequently shows an absence of amœbæ as well as of bacteria. Two or three days later amœbæ may be found in the pus draining from the abscess cavity.

Flukes, round worms, and whip worms may as a result of their wandering from the intestinal lumen cause abscesses.

Serious ulcerations may follow infection with the Guinea-worm.

Abscesses of filarial origin are to be thought of.

Focal Infections.—In recent years our attention has been directed to the importance of certain localized bacterial foci which may extend through hæmatogenous or lymph channels and give rise to various systemic or localized diseases. Most important of these diseases are various types of arthritis together with endocarditis, myocarditis and pericarditis. Next in importance are renal infections, chiefly of the glomerulonephritis type.

Cholecystitis, appendicitis, pancreatitis and various skin lesions may also have origin in a focal infection. The primary foci may be localized in any part of the body but those seated in the tonsilar, peridental membrane, nasal and accessory sinus tissues are the most common and important. Focal infections of the genito-urinary tract may also give rise to generalized conditions as is also true of such foci in the alimentary tract. In the tonsils we should particularly examine the material of crypts for various streptococci and likewise the bacterial flora of tooth abscesses or pyorrhœa alveolaris.

CHAPTER XXXIII

SKIN INFECTIONS

CULTURAL methods are as a rule to be preferred in the bacteriological examination of the skin.

This is best done by washing the surface to be examined with soap and water, in order to eliminate chance organisms which may have settled on the surface of the skin in dust or as a result of contact with material containing them. Scrapings are then made with a sterile dull scalpel, and this material is emulsified in a drop of sterile water in the center of a Petri dish. A tube of melted agar at 42°C. is then poured on the inoculated drop and, by mixing, the bacterial flora is distributed over the entire surface of the plate. Of the colonies developing on such plates probably 80% will be found to be staphylococci, and of these the greater proportion will be staphylococci showing white colonies.

Occasionally the aureus or citreus may be isolated.

Streptococci and colon bacilli are rarely found.

The *Staphylococcus pyogenes aureus* is the organism usually isolated from furuncles, circumscribed abscesses, and carbuncles.

Streptococci are the organisms to be expected in phlegmonous infections.

Cold abscesses, which are frequently due to tuberculous infection, are, as a rule, sterile.

Acne pustules may show staphylococci or the microbacillus of acne may be present.

The *Bacillus acnes* is a short broad bacillus often showing a beaded appearance when stained by Gram's method. It is Gram-positive. According to Hartwell it grows readily on glucose agar when cultivated anaerobically (Wright's method). Colonies appear in four to five days.

Sabouraud's medium for its culture is: Peptone 20 grams, glycerine 20 grams, glacial acetic acid 5 drops, agar 15 grams and water 1000 c.c. The bottle bacillus, which morphologically resembles a yeast, is considered to be the cause of dry pityriasis capitis. It may also be found in the comedones of children.

In the tropics, an organism which at times produces lesions similiar to impetigo and again pemphigoid eruptions and at other times widespreading erysipelatous conditions gives cultural characteristics similar to *S. pyogenes aureus*. It is probably only a virulent aureus. It has been described under the name of *Diplococcus pemphigi contagiosi*.

The *Staphylococcus epidermidis albus*, or stitch abscess coccus, is considered by Sabouraud to be the cause of eczema seborrhoicum.

It is in scrapings from the skin of lepromata that we find acid-fast organisms in the greatest profusion. In tuberculosis of the skin the tubercle bacilli are exceedingly scarce. Inoculation of a guinea-pig will probably give positive results with the tubercle bacillus. The leprosy bacillus is noninoculable for experimental animals.

Anthrax and glanders cause skin lesions which can only be surely diagnosed culturally or by animal inoculation.

Plague bacilli may be isolated from the primary vesicles appearing at the site of the flea bite.

Tropical phagedæna is thought by some to be due to a sort of diphtheroid organism. The organisms of Vincent's angina may cause tropical ulcer. Herpes zoster has been reported by Rosenow as most probably due to a streptococcus with special affinity for the ganglia and posterior roots.

The skin diseases due to fungi are discussed under that section. Of the skin affections caused by animal parasites, ground itch is the most important. This is a form of dermatitis due to the irritation set up by the hookworm larvæ penetrating the skin of the foot and leg.

The *Sarcopsylla penetrans* or jigger (sand flea) is an important agent in ulcerations about the foot.

Certain acarines cause skin lesions, as is also the case with the larvæ of certain flies.

The itch mite (*Sarcoptes scabiei*) is an important animal parasite of the skin.

The various lice, fleas and bedbugs are well understood as causes of skin irritation.

Filarial infections are also important especially the ulcers of the Guinea-worm, Calabar swellings of *F. loa*, the cystic tumors of *F. volvulus* and the varicose groin glands and elephantiasis of *F. bancrofti*.

Leeches, as *H. ceylonica*, may cause serious ulceration.

Oxyuris may cause a severe irritation about the region of the groin and inner surfaces of the thigh, and especially about the vulvar region of female children.

Gnathostomum siamense, a nematode with two lip-like structures and spine-like appendages covering its anterior one-third, has been found once in a tumefaction of the breast.

Plerocercoid larvæ of Dibothriocephalidæ have been found in the subcutaneous tissues.

Certain skin diseases, as Oriental sore and yaws, are protozoal in origin. The cutaneous lesions of uta or espundia are now known to be caused by a *Leishmania* as well as Oriental sore. These affections in the Central and South American countries are now known as American leishmaniases.

Dew itch or foot itch is caused by the penetration into the skin about the toes of the strongyloid encysted larva of the hookworm.

CHAPTER XXXIV

CYTODIAGNOSIS AND SPINAL FLUID EXAMINATIONS

THIS method of diagnosis is chiefly employed in the examination of cellular sediments of pleural, ascitic, and cerebrospinal fluid.

The fluids which pathologically collect in the serous cavities are divided into two classes, 1. the transudates, which form as the result of some circulatory inadequacy and 2. the exudates, which result from inflammatory processes.

Transudates have little or no fibrin and very few cellular elements and do not contain nucleo-albumin. Exudates contain nucleo-albumin and usually have a specific gravity above 1018, while that of the transudates is lower than 1018.

There are two simple methods for differentiating transudates and exudates. Moritz adds 2 drops of a 5% solution of acetic acid to the fluid to be tested. A heavy, cloud-like precipitate shows the fluid to be of inflammatory origin (an exudate). A transudate may produce a slight opalescence. Rivalta's test consists in dropping a drop of the fluid to be tested into a cylinder containing 2 drops of glacial acetic acid in 100 c.c. distilled water. A nebulous cloud as the drop of fluid sinks shows an exudate.

For pleural fluids we should receive the material in centrifuge tubes about one-fourth filled with 2% sodium citrate salt solution. This prevents clotting. Having thrown down the sediment, the supernatant fluid is poured off, and in its place a 1% aqueous solution of formalin is added. After mixing and allowing to stand for about five minutes, centrifugalization is again repeated and, pouring off the supernatant formalin solution, we make smears from the sediment. This is either stained by a Romanowsky method or, after fixing with heat (burning alcohol), the smear is stained with hæmatoxylin and eosin.

With ascitic fluid it is usually sufficient to centrifuge the fluid, then decant off the supernatant fluid and drain by means of a piece of filter-paper held at the mouth of the upturned tube. The sediment adheres to the bottom of the tube and is best emulsified with the small amount of fluid remaining by means of a bulb pipette. The material is sucked up, smeared out on a slide with a second slide as for blood and stained preferably with Giemsa after fixation. H. E. staining brings out mitotic figures best. If the fluid has coagulated it is best to take a little of the coagulum and stain it with neutral red as for vital staining. It is difficult to dissociate the cells from the clot. I now make it a rule to collect a portion of the pleural or ascitic fluid in citrated salt solution in order to prevent coagulation. The material is then centrifuged and after removal of supernatant fluid with a bulb pipette the cell sediment is drawn up and smeared out on a slide for cell or bacterial staining.

By making smears as for blood beautiful preparations may be obtained. I prefer Giemsa for differentiating cells and Gram's staining for bacteria.

The wet Giemsa method described for blood gives good results with puncture fluid sediments.

At the time of securing fluid for cytodiagnosis, cultures should be made on blood-serum for various pyogenic bacteria and, if tuberculosis is suspected, inoculation of a guinea-pig is indicated.

The interpretation of cellular sediments is more difficult than many books would indicate, there being many factors which tend to complicate the findings.

The polymorphonuclears in purulent fluids often show fatty degeneration, swollen and faintly staining nucleus or a breaking up of the nucleus into small deeply staining masses (nuclear fragmentation). Such fragments in the smear may be confusing. The endothelial cells often show fatty degeneration in the cytoplasm and we often note bacteria and other cells which have been phagocytized by them. Where proliferation of endothelial cells is going on actively the cells show a rather deeply staining cytoplasm as compared with the light staining cytoplasm of the cells in transudates. Some authorities attach importance to the Foulis' cells in connection with malignant processes in the peritoneum; often those associated with malignant types of ovarian cysts. Such cells are large, often multinucleated and may show appearance as if budding.

The following are the leading differentiations:

1. A smear showing almost entirely lymphocytes with a few red cells and very rarely a polymorphonuclear indicates a tuberculous process.

2. Where a pyogenic process is engrafted on a tuberculous one, we have still the red cells, some degenerated lymphocytes, and in particular polymorphonuclears showing fragmentation of their nuclei.

3. When a hydrothorax results from chronic heart or kidney disease, the characteristic cell is the endothelial cell, which greatly resembles a large mononuclear. These cells often are arranged in plaques.

4. Some authorities consider that the cancer cell can be recognized by its occurring in masses and having a markedly vacuolated cytoplasm. It has been claimed that they contain glycogen by which means we can distinguish them from endothelial cells which they so much resemble. If such cells should show mitosis the finding would be suggestive. For mitotic figures wet fixation with some bichloride fixative, with H. E. staining, is best.

Jousset introduced *inoscopy* as a means of diagnosing tuberculosis. The fluid was allowed to coagulate and was then digested with an artificial gastric juice. The

digested material was then centrifuged and the sediment examined for tubercle bacilli. This process does not seem to have met with much favor in this country. (Using sodium citrate obviates the necessity for digesting the coagulum).

The same points will hold for ascitic fluid as for pleural fluid.

Cerebrospinal Fluid Examinations

In taking cerebrospinal fluid for culture and cytodiagnosis we use a stout antitoxin needle without attaching a syringe. Aspiration is responsible for many of the ill effects of lumbar puncture.

The needle should be about 4 inches long for an adult. Sterilize the skin and needle as described for blood cultures from a vein. To make a lumbar puncture, place patient on left side with knees drawn up. A line at the level of the iliac crests passes between the third and fourth lumbar vertebræ. Select a point midway between the spinous processes of these lumbar vertebræ and enter the needle two-fifths of an inch to the right of this point, pushing the needle inward and upward. Collect the material in a sterile test-tube. Make cultures on blood-serum or blood-agar and then centrifugalize and examine the sediment as for pleural fluids.

Cell Count.—A method of examination considered by neurologists as of differential diagnostic value is to count the number of cells in a cubic millimeter of the cerebrospinal fluid. The technic is to use a gentian-violet-tinged 3% solution of acetic acid. This is drawn up to the mark 0.5, and the cerebrospinal fluid is then sucked up to 11. After mixing, the cell count is made with the hæmocytometer. Normally we have only one or two cells per cubic millimeter, but in tabes or general paresis this is increased to 50 or 100 cells (greatest at onset of disease).

It is advisable to make the cell count of the fluid as soon after obtaining it as possible, the cells tending to degenerate. It is customary to consider fluid containing blood as unsatisfactory for the cell count as well as for the globulin tests, but one can calculate the leukocytes due to blood content by counting the red cells and subtracting one leukocyte for each 750 red cells.

It is now generally recommended to make the spinal puncture with the patient seated on a stool with the shoulders inclined forward thus giving the greatest space between the spinous processes. After the puncture the patient should drink a glass or so of water and remain in bed for a day. In some clinics the subjects lie down for a few hours and then return to their homes.

In general terms, excluding syphilis, it may be stated that:

1. A lymphocytosis indicates a tuberculous or poliomyelitis process.

2. An abundance of polymorphonuclear and eosinophilic leukocytes indicates a meningococcic, streptococcic, influenza or pneumococcic infection. The fluid in (1) tends to be clear, that in (2) cloudy.

When the case is one of meningism there are very few cells. In poliomyelitis there is a cell increase of which 90% may be lymphocytes.

Trypanosomiasis gives a cellular increase very similar to syphilis.

In the work of the French Sleeping Sickness Commission five cells per cubic millimeter was taken as normal.

Miller gives the following table as to pleocytosis:

AVERAGE INCIDENCE OF LYMPHOCYTOSIS IN THE SPINAL FLUID
(Plaut, Rehm and Schottmuller)

Clinical diagnosis	Frequency	Remarks
Cerebrospinal lues.....	85–90%	Counts often over 100—may reach 1000 per c.mm.
Tabes dorsalis.....	90%	Counts usually under 100.
General paresis.....	98%	Counts average 30–60 cells per c.mm.
Secondary lues.....	30–40%	Moderate increase as a rule.
Multiple sclerosis.....	25%	Border-line counts.
Cerebral hæmorrhage....	Frequency is variable	Cellular increase is apt to be a very moderate one.
Cerebral tumors.....		
Sinus thrombosis.....		

Colloidal Gold Test (Lange's).—It is now generally accepted that this test is more diagnostic of general paresis than any other single test. The color changes in the first five tubes (1–10; 1–160) are so constant that the term "paretic curve" is applied to such findings. Of less diagnostic value are the so-called cerebrospinal lues curves where the color changes, though of less intensity than the paretic ones, are most marked in the third, fourth, fifth and sixth tubes (1–40 to 1–320). In various types of meningitis, other than luetic, the color changes are at times more marked in the tubes with the higher dilutions of spinal fluids (from 1–320 to 1–2560).

The paretic curve of the colloidal gold test generally runs parallel with a spinal fluid Wassermann and globulin increase. This agreement does not exist at all constantly for positive blood-serum Wassermann tests and increased cell counts.

It may be stated that this test is of more importance in paresis than any single one of the four reactions of Nonne, viz. (a) blood-serum Wassermann (b) spinal fluid Wassermann (c) globulin increase and (d) increased cell count of spinal fluid (pleocytosis). Of course, all of these tests should be carried out.

Test. Put 11 clean dry test-tubes in a rack and deposit in the first tube 1.8 c.c. of a 0.4% solution of sterile saline. Into the other 10 tubes put only 1 c.c. of the 0.4% saline. Into the first tube deliver 0.2 c.c. of spinal fluid and mixing thoroughly we have 2 c.c. of a 1–10 dilution. Withdraw 1 c.c. from the first tube and add to the 1 c.c. of saline in the second tube. This gives 2 c.c. of 1–20. Continue the process until the No. 1 to No. 10 tubes contain 1 c.c. quantities of the various dilutions from 1–10 to 1–5120.

Tube 11 contains no spinal fluid but only 1 c.c. of the saline and serves as a control.

To each of these 11 tubes add 5 c.c. of the colloidal gold reagent. The color changes are usually read after the tubes have stood over night at room temperature. The proper color of the control in tube 11 should be salmon red or old rose and the fluid should be perfectly transparent. When the color is changed in tubes containing dilutions of the spinal fluid we record one showing a bluish tint as 1. When the change is to a lilac we record it as 2. A distinct blue is marked as 3 and a pale blue as 4. When decolorization is complete there is the highest color change, which is noted as 5.

All glass-ware used in the test should be thoroughly washed in soap and hot water and then carefully rinsed with tap water. Next use the bichromate-sulphuric acid cleansing fluid, followed by most thorough washing in running water followed by distilled water.

In preparing the reagent a 2 liter glass beaker, following the above-described cleansing, is rinsed in double or triple distilled water, made with block tin condensing tubes and without rubber connections. Then fill the beaker with triple distilled water up to a ½ liter mark. Heat the water gradually to 60°C. Now add 5 c.c. of a 1% aqueous solution of Merck's yellow crystalline gold chloride and 3½ c.c. of a 2% aqueous solution of desiccated potassium carbonate. Continue the heating of the solution, which should remain clear, to 80°C., then add 5 drops of a 1% aqueous solution of oxalic acid, stirring all the time. The solution should be colorless after adding the oxalic acid. When the temperature reaches 90°C. remove the flame and add drop by drop 5 c.c. of 1% formalin solution, stirring continuously. Should a pink color show itself before all the formalin solution has been added stop the further addition. It soon assumes the required shade and when cool should be perfectly transparent and of an old rose or orange-red color.

Numerous trials were made at the U. S. Naval Medical School and the following method has been adopted.

Glassware.—New glassware should be used, and of course should not be used for any other purpose. It is first cleaned with hot soapy water then rinsed with aqua regia. After this, all flasks, pipettes, etc., are completely filled with tap water several times to drive out all traces and fumes of the aqua regia and then rinsed with distilled water, and just before use with triple distilled water. We found that the following glassware is all that is necessary in the preparation of colloidal gold: 3–100 c.c. volumetric flasks, 1–100 c.c. pipette, 1–10 c.c. pipette, 1–10 c.c. graduated pipette, 1–5 c.c. graduated pipette, and several 2 liter Erlenmeyer flasks. Each pipette should be reserved for only one solution and they should not be interchanged. If

this rule is followed, a simple rinsing with triple distilled water before making each solution is all that is necessary and the initial cleaning with aqua regia need only be repeated at long intervals.

Water.—All water used in the preparation of the stock solutions and the reagent itself must be triple distilled. A block tin condenser can be made from a piece of block tin tubing about 60 cm. long, bent in the form of an inverted U with one long arm and one short arm. The short arm is passed through a tinfoil-covered cork into the distilling flask and the long arm is surrounded by a glass or metal cooling jacket through which water circulates. The lower end of the tube passes into a flask placed to catch the distillate.

To the second distilled water is added a small quantity of sodium carbonate (about 1 gm. per liter). We believe this to be very important, as it frees the water from all traces of ammonia that may be present due to absorption of the gases in the laboratory, and as a further precaution against impurities, the first 50 to 100 c.c. of the third distillate is discarded.

Solutions Required.—(1) Gold chloride (Merck's), 1 gm., water (triply distilled) up to 100 c.c. (2) Potassium carbonate, 2 gm., water (triply distilled) up to 100 c.c. (3) Formalin (40% Formaldehyde) 2.5 c.c., water (triply distilled) up to 100 c.c.

Preparation of the Colloidal Gold Solution.—Place 1000 c.c. of triply distilled water in a 2 liter Erlenmeyer flask. (A large flask facilitates vigorous agitation at the end of the procedure). Add 10 c.c. of 1% gold chloride solution and 7 c.c. of 2% potassium carbonate solution. Place over a Bunsen burner and heat rapidly to boiling. When the boiling point is reached, as evidenced by the rising of bubbles, remove from the flame and with constant agitation add 4 c.c. of 1% formaldehyde (2.5% formalin solution). Continue to agitate vigorously and the solution will be seen to assume a faint bluish tinge and pass rapidly through a deep amethyst to a beautiful red color which indicates that reduction is complete. This is the final product, and it should conform to the following requirements:

1. The solution must be absolutely transparent and preferably of a brilliant red-orange or salmon red color.

2. Five c.c. of the solution must be completely precipitated by 1.7 c.c. of a 1% solution of sodium chloride in the time interval of one hour.

3. The solution must be neutral in reaction.

4. It must give a typical reaction with a known paretic cerebrospinal fluid.

5. It must produce no reaction greater than a No. 1 with known normal cerebrospinal fluid.

If a solution results that is unsatisfactory in any of these particulars, it should be discarded, and after a careful and thorough recleaning of all glassware, another solution made up. This procedure in our experience has always resulted in a satisfactory solution and takes less time and is less trouble than trying to "correct" a poor solution.

Globulin Increase Tests.—The test generally used is *Noguchi's butyric acid* one. Deliver into a small test-tube 0.5 c.c. of a 10% solution of butyric acid in 0.9% salt solution. Then add 0.1 c.c. of spinal fluid. Bring to a boil over a flame and add 0.1 c.c. of N/1 NaOH solution.

If there is a considerable increase of globulin a flocculent precipitate appears in a few minutes or at any rate in one or two hours. Fluids with a normal content or only slight increase only show a slight opacity.

The odor of the butyric acid is very objectionable and in our laboratory we use the *Ross-Jones* method. In this one deposits in a small tube about 1 c.c. of saturated solution of ammonium sulphate. On the surface of this column we deposit 1 c.c. of spinal fluid. If globulin increase is present a turbid ring appears within a few seconds at the junction. Normally there is no sign of a ring. This test is a modification of Nonne's Phase I reaction.

A test that is not in general use is strongly recommended by Miller. It is known as *Pandy's test.* To carry it out prepare a saturated solution of carbolic acid crystals in distilled water. Place 1 c.c. of this reagent in a small test-tube and add 1 drop of spinal fluid. In a normal fluid only the faintest opalescence is observed, but in a fluid with globulin increase a smoke like white cloud develops instantly where the drop comes in contact with the reagent. Miller gives the following table as showing the average frequency of the various reactions in syphilis of the central nervous system.

SHOWING THE AVERAGE FREQUENCY OF THE VARIOUS REACTIONS IN SYPHILIS OF THE CENTRAL NERVOUS SYSTEM

	Paresis	Tabes dorsalis	Cerebrospinal syphilis
Blood Wassermann.....	98–100%	70%	70–80%
Spinal fluid Wassermann....	97%	60–80%	85–90%
Pleocytosis.....	98%	85–90%	85–90%
Positive globulin test.....	100%	90–95%	90–95%
Colloidal gold test.....	98–100% Paretic curves	85–90% Luetic type of curve	75–80% Luetic curve

Poliomyelitis in addition to small mononuclear increase together with rather characteristic large mononuclear cells shows a globulin increase.

Urea Content.—Any excess of urea in the cerebrospinal fluid is a sure sign of renal inadequacy.

Normally the urea content of cerebrospinal fluid is only 0.006%, according to Canti. Cases of true uremia, and not renal disease associated with cardio-vascular disease, show from 0.1 to 0.6%. Cases showing over 0.3% rarely recover. The estimate may be made from 5 c.c. of spinal fluid by the urease method.

CHAPTER XXXV

RABIES, SMALLPOX, VACCINIA AND THE FILTERABLE VIRUSES

RABIES is a disease of dogs and wolves, but is communicable to man and domesticated animals. The virus, whatever it may be, resides in the saliva and nervous structures, It is destroyed by a temperature of 50°C. In man the period of incubation is usually from three weeks to three months, but may be shorter or may extend over one year.

Bites about the face and those with marked lacerations are particularly serious. Bites of rabid wolves give about four times as great a mortality as those of dogs. In the dog there are two types of the disease—dumb rabies and furious rabies.

By inoculating rabbits subdurally with an emulsion of the brain or spinal cord of a rabid animal, and successively the medulla of this rabbit subdurally into other rabbits, we finally so increase the virulence of the infection that rabbits die in six days. Beyond this it is impossible to increase the virulence and it is termed "fixed virus." The pathogenic power of this virus is also changed so that it is not apt to cause rabies if injected subcutaneously. To attenuate this virus the spinal cord of the rabbit is removed and is dried over caustic potash at a temperature of 23°C. The cord is divided into segments about 1 inch in length. Drying for about fifteen days seems to entirely destroy the virus.

To prepare the material for prophylactic injections a small portion of the cord is emulsified with normal salt solution and injected subcutaneously. The German method is to commence with a cord that has been desiccated only eight days. At first injections are given daily, and it is possible to inject three days' cords by the sixth day. The immunity is "active" and the immunizing agent is a "vaccine." Like vaccine virus the product can be preserved (for probably a month) by the use of glycerine so that it is now possible to send the material for inoculation from the laboratory preparing it.

The treatment lasts for about twenty days.

There are other methods of treatment as follows:

1. **The Harris Method.**—In this the brain and cord are ground up with CO^2 snow and the frozen tissue dried over H^2SO^4. The process of drying lasts about two days and the virulence of the virus is reduced one-half. The potency of the virus, when kept at 0°C., holds for six months at least.

2. **The Cumming Method.**—In this the brain is emulsified in saline and dialyzed. In this method the virus is so attenuated that injections do not produce rabies on intracranial inoculation.

3. **In the Hogyes method** the fresh virulent cord is injected but so diluted in strength that it acts as does an attenuated virus.

In the diagnosis of rabies in dogs it is preferable to preserve the animal so that the development of the symptoms may be observed.

In case the dog has been killed, it may be possible to make a diagnosis by means of the Negri bodies. These are round or oval bodies from

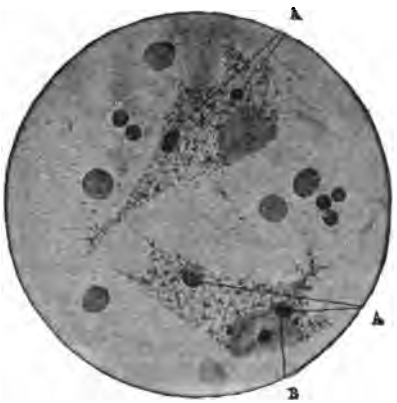


FIG. 139.—Two nerve cells of hippocampus major (smear preparation) showing *Negri bodies. A, Negri bodies; B,* inner bodies within the *Negri bodies. (After Reichel, American Veterinary Review.)*

1 to 20μ in diameter, which may be found in the nerve-cells, especially those of the cornu ammonis (Hippocampus major).

These bodies were first described by Negri in 1903. In street rabies large ameboid forms from 18 to 23μ may be found, while in the nerve tissues of animals with "fixed" virus only minute forms, 0.5μ or less, may be detected. The fact that the virus will pass through a Berkefeld filter is no argument against its protozoal nature. Calkins considers it to be of rhizopod affinity. The name *Neuroryctes hydrophobiæ* has been given it. The bodies are present four to seven days before the onset of symptoms. They may be demonstrated by staining smears of gray brain substance by some Romanowsky method, especially by the Giemsa stain. The smears should be made by mashing the thin slice of gray matter taken from 1. Cornu ammonis, 2. Region of fissure of Rolando—in dog crucial sulcus—or 3. Cerebellum, with a cover-glass against the slide. Afterward the cover-glass is gently drawn along the slide.

The smear on the slide is then fixed in methyl alcohol for two or three minutes, washed with water and covered with a stain made by adding 3 drops of Sat. alc. sol. of basic fuchsin to 10 c.c. of distilled water and then adding 2 c.c. of Löffler's methylene-blue solution. The stain on the slide is then steamed gently and afterward washed with water and dried.

As their relation to the nerve-cell is more or less disturbed by such a method it is preferable to fix brain tissue from the region of the cornu ammonis for five to seven hours in Zenker's fluid, then to imbed in paraffin and make sections. These are stained with Giemsa's stain and the Negri bodies are brought out as lilac-red bodies in the blue cytoplasm of the nerve-cells. It is necessary to differentiate in 95% alcohol.

In the Lentz method the 3μ sections, after removal of the paraffin, are flooded with absolute alcohol. They are then stained with a ½% solution of eosin in 60% alcohol for one minute. Wash in water and next stain for one minute in Löffler's methylene blue. Again wash in water. Apply Lugol's solution to the section for one minute and then differentiate alternately in methyl alcohol and water until the section is pink. After washing in water, again stain with Löffler's blue for one-half a minute, then wash in water and dry carefully with filter-paper. Now differentiate in alkaline alcohol (1 drop of a 5% solution NaOH in 30 c.c. absolute alcohol) until the section is pink, then quickly differentiate in acid alcohol (1 drop 50% acetic acid in 30 c.c. absolute alcohol) until a slight blue outline to the ganglion cells is obtained. Treat rapidly with absolute alcohol and xylol and mount in balsam. The Negri bodies show as light carmine pink bodies on the light blue ground of the ganglion cells. In the interior of the pink bodies dark blue dots or rings may be observed.

This method can also be used for brain smears.

In addition to examining for the Negri bodies, a rabbit may be inoculated subdurally with a sterile salt-solution emulsion of the medulla of the dead dog.

If the brain and medulla of the dog are to be sent to a laboratory for examination they should be packed in ice or placed in glycerine. Take of glycerine one part and one part water. Sterilize the diluted glycerine by boiling, allow to cool, and drop the pieces of brain tissue into this. This does not kill the virus.

When from advanced putrefaction, or otherwise, the Negri bodies cannot be found the changes in the Gasserian ganglia may give a diagnosis. In typical lesions the ganglion cells are more or less completely destroyed and replaced by cells of other types.

When a person is bitten by a dog suspected of being rabid the following simple measures should be instituted. The dog should be kept under observation in a safe quiet place and will show clinical evidence of rabies within five days and will die shortly afterward in case rabies exists. When the animal dies the head and several inches of the neck should be removed and packed in ice and sent to the nearest laboratory.

Antirabic serum has been prepared by injecting sheep with emulsions of rabbits' cord and brain—at first intravenously, then subcutaneously.

The thorough cauterization of the dog-bite wound with pure nitric acid, as soon as possible after the bite, is imperative even when the Pasteur treatment can be given later.

Smallpox.—The etiology of this disease is very obscure, the virus being grouped under the Chlamydozoa. Smallpox and vaccinia are often classed as filterable viruses. Park, however, was unable to pass the virus of vaccinia through a Berkefeld filter with 40 pounds pressure; the failure may have been due to lack of sufficient dilution. They did find that the virus would pass through the finest filter-paper.

In 1892 Guarnieri noted cell inclusions in the cornea of rabbits inoculated with smallpox and under the name *Cytorrhyctes variolæ*, Councilman has described what he regards as a protozoon invading cell nuclei. Certainly ordinary bacteria are not concerned in the etiology of smallpox. The virus is not only contained in the skin lesions but also in nasal and buccal secretions, the disease being communicable before the eruption appears. The period of incubation of variola vera is very constantly twelve days, while that of variola inoculata (a method of prophylaxis by inoculating discharges from a vesicle or pustule which preceded the present method of vaccination) is eight days. Monkeys are quite susceptible to both smallpox and vaccinia and rabbits to corneal inoculations.

Cutaneously the rabbit shows a typical eruption after vaccination, but does not show characteristic lesions after smallpox inoculation.

It is usually accepted that vaccinia is simply a permanently modified smallpox resulting from animal passage and it is stated that repeated passage of smallpox virus through calves produces vaccine virus. Inoculation of calves with smallpox virus is a most uncertain procedure and Park states that he has been unable to obtain success after many such experiments.

Of great practical importance is the differentiation of smallpox and chicken-pox. In efficiently vaccinated persons the cutaneous inoculation of material from the suspicious vesicle will give rise to a skin reaction similar to the Pirquet Tb. one, occurring within twenty-four hours. If the vesicle were of chicken-pox no reaction occurs. Heating the material to 60°C. for thirty minutes eliminates all danger and does not interfere with the reaction. (*Tièche.*)

The best and most convenient method of vaccination is by some intracutaneous method as this does not leave an abrasion to become infected and the patient cannot wipe away the virus to avoid a take. One of these methods is the multiple puncture one.

Vaccinia.—Vaccinia is a disease produced artifically by the injection of vaccine virus obtained from the calf. The material for vaccine is

taken from vesicles about one week after the inoculation. The most potent material is in the pulp at the base of the vesicle and not in the lymph which exudes from the vesicle. The pulp is ground up and mixed with an equal amount of glycerine, which acts not only as a preservative but as a mild antiseptic for nonsporing bacteria. The calves are autopsied after the pulp has been curetted from the inoculated skin of the abdomen to be sure that no disease exists in the calves. The virus is afterward tested for pus organisms, tetanus, and foot and mouth disease.

Very important is the test for tetanus. Cultures are grown anaerobically for six days, then filtered and the filtrate inoculated into guinea-pigs and these latter watched for ten days to note evidence of tetanus.

If found free from any harmful germs the vaccine is then tested upon children for potency. Incision is the proper method of vaccination, as by scratching with a needle, two lines 1 inch long and 1 inch apart. Scarification should never be practised.

Guarnieri in 1892 first observed small bodies near the nucleus of infected epithelial cells. He called them *Cytorryctes vaccinia*. Calkins regards these bodies as well as the Negri bodies as being rhizopods and the distributed chromatin as idiochromidia (granules of nuclear chromatin within the cytoplasm).

The Filterable Viruses

The first disease of which the virus was found to be capable of passing through the finest porçelain filter was that of foot and mouth disease (Löffler and Frosch, 1898).

The filter which is ordinarily used for testing for the passage of such disease agents is the Berkefeld filter, one made of diatomaceous earth. The filter should be new and sterilized before use. The material should be diluted with saline before filtering. One may use slight suction from a filter pump. The filtration should occupy only a short time, not exceeding two hours.

Of the infections belonging to man, in which such a passage of blood or serum through the pores of a porcelain filter, capable of keeping back even such a small bacterial organism as that of Malta fever, but which does not hold back their virus, we have the following: foot and mouth disease, trachoma, molluscum contagiosum, vaccinia, variola, rabies, typhus fever, measles, scarlet fever, yellow fever, dengue, Papataci fever, poliomyelitis and coryza. It has been suggested that the virus of cerebrospinal meningitis may be a filterable one.

Hog Cholera Virus.—Very interesting is the history of the virus of hog cholera or swine fever. This was supposed to be due to an organism of the hog cholera group, *B. aertrycke* (identical with *B. choleræ suis* and *B. suipestifer*). This organism belongs to the "enteritidis" group and is more common as a cause of food poisoning in man than the better known Gaertner bacillus. Recently the group of organisms, including the paratyphoid B., but not A, as well as the hog cholera group, has been designated the *Salmonella* group. It is now known that the cause of this most important fatal disease of swine is a filterable virus.

This virus shows remarkable powers of resistance to external influences, thus it can be kept for months in animal tissues. It is not destroyed by drying and withstands a temperature of 58°C. for two hours but not one of 72°C. for one hour. Cell inclusions have been found in smears from the conjunctivæ of hogs sick with the disease. See Chlamydozoa. A very valuable prophylactic but not curative serum is found in the serum of animals recovering from the disease or in those immunized.

There are many other diseases of this nature which are important among the domesticated animals, such as pleuropneumonia of cattle, African horse sickness and hog cholera. The viruses of pleuropneumonia of cattle and poliomyelitis have been obtained in artificial cultures. Some of these viruses seem related to bacterial infections and others to protozoal ones. These viruses differ as to method of transmission, pleuropneumonia of cattle being transmitted by inhalation, rabies and vaccinia by the cutaneous atrium, hog cholera by ingestion and many of those supposed to have protozoal affinities, as yellow fever, Papataci fever and horse sickness by mosquitoes.

As a rule these viruses are destroyed by a temperature of 55°C. in a few minutes.

CHAPTER XXXVI

DISEASES OF UNKNOWN OR NOT DEFINITELY DETERMINED ETIOLOGY

Of Temperate Climates

Acute Articular Rheumatism.—Various bacteria have been reported as cause. The organism which seems the most probable cause is the short chain coccus, *Micrococcus rheumaticus* of Triboulet and others. Inoculations of this streptococcus cause polyarthritis and pericarditis. Poynton and Paine have cultivated the organism from the cerebrospinal fluid in three cases where chorea was present.

The Common Cold.—Of all the diseases common in man this condition has been surrounded by greater etiological and epidemiological obscurity than any other.

We are inclined to believe that the common cold (coryza) sets in when our resistance is lowered by alimentary tract disturbances, from exposure to variations in temperature, or following refrigeration and fatigue. Of course, many have held that the common cold was "catching" but the evidence offered in support of such a view has been academic.

Many bacterial organisms have been suggested as causative such as *B. coryzæ segmentosus*, hæmolytic and viridans types of streptococci, *M. catarrhalis*, etc. In 1914 Kruse brought forward evidence to prove that the etiological factor in coryza was a filterable virus. Quite recently Foster has conducted experiments in which, by using the nasal discharge from typical coryza cases, diluting it with 10 or 15 times its volume of saline, then passing through a small Berkefeld filter and instilling 3 to 6 drops of the filtrate into the nasal cavity of 10 well men he produced typical coryza in nine of these men in from eight to thirty hours. Cultures were made from the filtrate following Noguchi's spirochæte culturing method. The culture medium surrounding the piece of sterile tissue showed turbidity in from forty-eight to seventy-two hours and dark-field examination showed myriads of extremely active bodies which were thought to possess true motility rather than Brownian movement.

Filtrates from these cultures were instilled into the nasal cavity of 11 men and after a period of incubation of from eight to forty-eight hours all came down with coryza.

Epidemic Poliomyelitis.—Material from the cord of child with the disease when injected subdurally, intravascularly, or into the peritoneal cavity of monkeys produced the disease in the animals inoculated. The virus has been passed through three generations of monkeys (Flexner).

The virus has been found in the brain, spinal cord, mesenteric and salivary glands of monkeys and may remain in the nasal mucosa of monkeys as long as five months. This would indicate the existence of human chronic carriers. With the possible exception of the rabbit only man and the monkey are susceptible. This would indicate that the virus is directly transferred from man to man. The virus is highly resistant to drying and light. It will remain alive for months in dust. It is not sterilized by pure glycerine during many months of contact. It is possibly transmitted by a biting fly, *Stomoxys calcitrans*. Against this is the fact that the virus has not been found in the blood.

Flexner and Noguchi have recently cultivated the virus of poliomyelitis by employing ascitic fluid to which had been added a fragment of sterile rabbit kidney and nutrient agar, this culture medium being covered with a layer of paraffin oil. The growth is obtained under anaerobic conditions. The minute colonies are composed of globular or globoid bodies from 0.15 to 0.3 micron in diameter. These bodies may be single or in chains or in masses. In older cultures bizarre forms are obtained. Monkeys have been inoculated with the cultures.

Rosenow considers streptococci having an affinity for the central nervous system as the excitants of the disease.

Foot and Mouth Disease.—Due to an ultramicroscopic organism.

This is a highly contagious disease of cattle characterized by the appearance of vesicles in the mouth and about the feet of cattle. Man rarely contracts the infection through drinking the milk of infected animals. This disease is of great interest as having been the first of the filterable virus diseases to have been discovered (Löffler and Frosch in 1898).

Measles.—Cause entirely unknown. Hektoen has shown that blood contains the virus.

Anderson has found that the virus of measles can pass through a Berkefeld filter and loses its infectivity after heating for fifteen minutes at 55°C. In infecting monkeys it was found that the blood of patients with measles was infective only

just before and for about twenty-four hours after the appearance of the eruption. Mixed nasal and buccal secretions were infective for monkeys for about forty-eight hours from the time of the eruption. The scales from desquamating cases were not capable of infecting monkeys hence it was thought that measles was not contagious during the period of desquamation.

Mumps.—Herb has implicated a diplococcus. Inoculations into Stenson's duct of monkeys successful.

Rabies.—Probably the Negri bodies.

Roetheln (German Measles).—Nothing known.

Scarlet Fever.—Streptococci seem most probable cause (*S. anginosus*). Mallory has implicated epithelial protozoa.

Dohle has reported the rather constant finding of basophilic round or oval inclusion bodies in the polymorphonuclears. These findings, which are brought out in stained films, are present only during the first few days of the attack of scarlatina. Other workers have found these bodies almost as constantly present in diphtheria as in scarlet fever. They have also been found in other acute diseases than diphtheria. Klemenko has obtained streptococci from the blood in only about 2% of cases of scarlatina. Quite recently Mallory has reported diphtheroid organisms as the cause.

Smallpox and Vaccinia.—Guarnieri and Councilman have implicated epithelial protozoa.

Spotted Fever of the Rocky Mountains.—Supposed to be due to an unknown protozoon transmitted by a tick, *D. andersoni.*

This disease is especially prevalent in the Bitter Root Valley of Montana and to some extent in the mountains of Idaho. It is an acute febrile affection with a tendency to stupor. The eruption, which appears about the third to fifth day, is not unlike that of typhus fever and tends to become hæmorrhagic. Gangrene of penis or scrotum may appear. It is transmitted by a tick, *Dermacentor andersoni* (*D. venustus*) which lives on domesticated animals of the region. Destruction of ticks which attach themselves to sheep by dipping has been proposed as a measure for eradication of the disease. Ricketts found that the reservoir for the virus is to be found in ground squirrels, chipmunks, mountain rats, etc., and that ticks feeding upon them become infected and so transfer the disease to man. Guinea-pigs are susceptible as is also the monkey. Ricketts noted certain chromatin staining bacteria in man and in eggs of infected ticks as possibly playing a part in etiology. Quite recently Wolbach has reported the finding in infected guinea-pigs of organisms, possibly bacterial, showing granular and lanceolate forms. They are particularly abundant in the endothelial cells of blood-vessels. They are from ½ to 1 micron long by about ⅓ micron broad. Ricketts stated that his organisms were about the size of *B. influenzæ* and showed as two lanceolate-shaped bodies. Wilson and Chowning, in 1902, reported the finding of piroplasm-like organisms in the blood of the disease. Ricketts proved that the virus was not filterable.

Trachoma.—This contagious form of granular conjunctivitis is supposed to be due to chlamydozoa or inclusion bodies and is classed as one of the filterable viruses. The relation of the trachoma bodies to the Koch-Weeks bacillus is discussed under that organism.

Trench Foot.—This is a condition caused by defective circulation in the feet. It is brought about by prolonged standing in wet trenches during cold although not necessarily freezing weather. The feet are swollen and usually shown a reddish congestion although they may be white. There is a resemblance to frost bite and Raynaud's disease. The feet may show a gangrenous appearance. There was an idea that the condition might be due to a fungus infection but this view is no longer held.

Trench Nephritis.—Nephritis, showing œdema, increased blood pressure, headaches and lumbar pain has been rather common in those exposed to the wet and cold of the trenches. It is relatively mild in type and the prognosis is good. There has been a view that it might be connected with a spirochæte infection but such etiology is not generally accepted.

Typhus Fever.—It has been suggested that the cause may be a protozoon transmitted by vermin.

Recent work by Anderson and Ricketts has shown that the blood of human cases is infective for monkeys. The virus does not seem to pass through a Berkefeld filter and the epidemiology points to the body louse as the transmitting agent. Nicolle reported the filterability of the virus.

Plotz has isolated a Gram-positive pleomorphic bacillus from the blood of typhus patients as well as from the blood of guinea-pigs and monkeys infected by injections of typhus blood. It is most abundant in blood taken four or five days before the crisis. It only grows anaerobically and grows best in ascitic fluid sterile tissue media. Morphologically it shows curved, straight and coccoid forms. The rods are about 1.5 micron long. The serum of convalescents shows complement-fixation bodies as well as agglutinins. The organism has been named *B. typhi exanthematici.*

Hort states that only blood recently taken from typhus patients will cause the disease in monkeys while the same blood which has been incubated several hours or days fails to produce the disease. Others, as well as Hort, doubt the etiological relation of the organism of Plotz to typhus fever or the mild form of the disease as seen in New York City and there known as Brill's disease. Tabardillo or Mexican typhus is the same as typhus.

Varicella.—Entirely unknown.

Whooping-cough.—Influenza-like bacilli have been implicated. Bordet-Gengou bacillus.

Of Tropical Climates

Ainhum.—A disease characterized by a constricting fibrous ring, especially of little toe, often leading to spontaneous amputation.

Beriberi.—Various microörganisms and food factors suggested. A form of multiple neuritis, occurring chiefly in countries where rice is the staple food, characterized by œdema and marked cardiac and respiratory embarrassment. The vagal involvement produces grave symptoms. Rice from which the pericarp has been largely removed, polished rice, implicated.

Prior to the investigations of Fraser and Stanton the importance of the rice factor in the etiology of beriberi was insisted upon by Braddon who thought that a poison was elaborated by some organism which poison was contained in the beriberi producing rice. This development was thought to occur in rice stored in damp places, but Vedder has shown that storing undermilled rice in a damp place for a year does not cause it to lose its anti-beriberi producing properties. The work of Eijkman in showing that polyneuritis could be produced in fowls by feeding them on polished rice and prevented when a diet of rice polishings was added to the neuritis-producing rice opened the way for a vast amount of experimental work. As regards the nature of the neuritis-preventing substance in the rice polishings it was soon found that it had no relation to the phosphorus content. Funk has isolated a substance he calls *vitamine*, a pyramidine base precipitated by phosphotungstic acid, which is present in rice in the proportion of 1 to 100,000 and seems to possess extraordinary curative properties in polyneuritis gallinarum. Heart muscle, egg yolk and yeast are rich in this anti-neuritis substance, which is also present in lentils and barley. Schaumann considers malt as richer in the anti-neuritis vitamine than any other article of diet, rice bran coming next. Many think that vitamines have not as yet been separated but that they are intimately combined with some mother substance in the food. There is, in all probability, a large number of vitamines present in various animal and vegetable foods, the deficiency of which in a diet may lead to vague disorders or to well-recognized diseases, such as scurvy, ship-beriberi, beriberi or pellagra.

Schaumann considers the curative principle to be of the nature of an activator. An increase in the ingestion of carbohydrates and necessarily in the vitamine as well seems to produce neuritis more rapidly than where a smaller amount is given, this indicating the importance of these vitamines in carbohydrate metabolism.

In epidemics of beriberi it has been observed that those who eat most rice are more often attacked, thus men more frequently than women. A temperature of 120°C. destroys the vitamine. Owing to the absence of rice as a constituent of other than slightest importance in the dietary of Brazilian cases of beriberi, as well as from numerous reports of the occurrence of the disease in nonrice-eating persons, the view

that is now entertained is that not only polished rice, but any predominating carbohydrate article of diet, which is deficient in the neuritis-preventing substance, can produce beriberi. Wellman and Bass have shown that such articles of diet as sago, boiled white potatoes, corn grits and macaroni practically parallel polished rice in the production of polyneuritis in fowls.

Blackwater Fever.—Considered as a malarial disease, but thought by some to be possibly caused by a protozoon—a *Babesia* (*Piroplasma*).

A disease usually occurring in patients with a malarial history and characterized by rapid febrile onset, early jaundice, asthenia, pain in loins and the pathognomonic hæmoglobinuria.

Dengue.—Supposed to be due to a protozoon transmitted by *Culex fatigans*. A disease characterized by sudden onset, high fever for three or four days, pains in the postorbital regions, back and about joints.

A remission occurs on the third to fifth day followed by a secondary rise of temperature and a measles-like eruption. Leukopenia and reduction in the percentage of polymorphonuclears. Virus exists in the blood and is filterable.

Goundou.—Symmetrical bony tumors of nasal processes of superior maxillary bones.

Oroya Fever.—A disease with a fever characterized by a profound involvement of the bone marrow producing very rapidly an anæmia resembling that of pernicious anæmia. Pains of bones and joints marked. See Verruga.

The disease is chiefly found in towns situated in narrow, wind-protected valleys of the West side of the Andes, at elevations of from 3000 to 9000 feet. Townsend has suggested that a species of *Phlebotomus*, which is very prevalent, may be the transmitting agent.

Barton isolated a paratyphoid bacillus from the blood of a patient, besides which other bacteria have also been isolated. In 1909, Barton noted certain rod-like organisms in the red cells of Oroya fever patients which he considered protozoal in nature.

Strong and his colleagues found in the blood of Oroya fever cases rod-shaped forms in the red cells, varying from 1 to 2 microns in length, the red cells containing from 1 to 30 of these elements.

Intravenous inoculation of blood containing these elements into monkeys and rabbits was negative in result. These organisms were considered as intermediate between bacteria and protozoa. They are closely related to *Grahamella* and the Harvard commission has proposed the name *Bartonella bacilliformis*.

Pellagra.—This disease is characterized by (1) a sprue-like stomatitis and disorders of alimentary canal, (2) an erythema usually limited to parts exposed to the sun and characterized by marked symmetry and striking delimitation from the sound skin and (3) various neurological manifestations and a toxic psychosis which may go on to confusional insanity. The disease is characterized by annual recurrences in the spring with improvement in the winter. The views as to etiology which consider the causative agent as a protozoon, possibly transmitted by a *Simulium* or by *Stomoxys* are now historical.

The maize ideas of etiology are now considered as having a bearing only in connection with the larger question of vitamine deficiency in cereals in general. Alessandrini has recently brought forward the colloidal silica etiology.

These views are that colloidal silica in water is responsible for the disease. Voegtlin noted the great amount of aluminium in certain vegetables and suggested this as the toxic causative substance. A mixture of colloidal alumina and silica in water is supposed to be operative as well as silica alone. Against the colloidal silica hypothesis is the statement of Sandwith that the water of the Nile, the drinking water of Egypt, is low in colloidal silica content.

The present trend of thought in connection with pellagra is that it is a food deficiency disease connected with a deficiency in vitamines necessary for normal metabolism (see beriberi).

In February, 1915, Goldberger started a "pellagra squad," consisting of 11 prisoners on a diet of wheat flour (patent), cornmeal, corn grits, corn starch, polished rice, granulated sugar, cane syrup, sweet potatoes, fat fried out of salt pork, cabbage, collards, turnip greens and coffee. Baking powder was used for making biscuits and corn bread. The food value of each man's diet averaged 2952 calories.

A control was carried out with prisoners on a normal diet. The experiment was continued until Oct. 31, 1915. Of the 11 volunteers on the excessive carbohydrate diet six developed symptoms. Loss of weight and strength and mild neurasthenia were early symptoms. Definite cutaneous manifestations appeared only after five months. The skin lesions were first noted on the scrotum, later appearing on backs of hands in two cases and back of neck in one case.

Sixteen volunteers, working under Goldberger, tried to infect themselves with blood, nasopharyngeal secretions, epidermal scales, feces and urine from pellagrins. Various atria of infection were tried according to material; blood by intramuscular injection, excreta by mouth. After a period of six months all the subjects of the experiments remained well. *This evidence is certainly against the infectious nature of the disease.*

Just as with rice so does excessive milling of wheat get rid of vitamines, therefore bread made from highly milled flour is dietetically deficient.

Again, as brought out by Voegtlin, alkalies tend to destroy any remaining vitamines in such bread. The practice of using sodium bicarbonate in preparation of bread is a further factor in the food deficiency problem. With the use of baking

powder or buttermilk the alkaline carbonate of soda is neutralized so that there is no destructive effect on vitamine content.

Notwithstanding the above evidence as to food deficiency etiology it must be remembered that McNeal and his colleagues on the Thompson-McFadden pellagra commission, as well as other authorities on this disease, insist upon a probable infectious agent as cause.

Rat-bite Disease.—A disease caused by the bite of rats. Rather common in Japan. Five weeks after bite when wound has healed, high fever sets in, cicatrix becomes inflamed with lymphangitis and swollen glands. The fever falls in a few days to be succeeded by other febrile paroxysms. An erythematous eruption accompanies the second paroxysm.

Supposed by Ogata to be due to a protozoon, but recent work by Schotmüller, in 1914, has shown that the cause is a *Streptothrix, S. muris ratti.* This finding has been corroborated by Blake. The organism first invades the lymphatic structures and then the blood, giving a septicæmia. Various organs are later involved. Blake's case developed a powerful agglutinin for the specific *Streptothrix.*

Sprue.—A form of chronic diarrhœa characterized by diaphanous thinning of gut and ulcerations of buccal cavity.

Kohlbrugge found organisms resembling *Oidium albicans* in the intestines, œsophagus and tongue. He found similar organisms in the stools and tongue scrapings of cases of sprue. Beneke found bacilli in the tongue, œsophagus and intestines and considered these as causative, regarding the thrush-like membranous deposit as connected with the cachectic state and not causative.

Bahr is inclined to believe that *Monilia albicans* (*Oidium albicans*) is the cause as he found these saccharomycetes in the deep layers of the tongue, in the mucoid coating of the intestines and in the deposit in the œsophagus. He thinks it the ordinary thrush species which may take on greater virulence in the tropics. Ashford states that he has found a species of *Monilia*, different from that of thrush, almost constantly in tongue scrapings and stools of sprue cases and he regards this species as the cause of sprue. He states that this organism is common in Porto Rico bread and thinks it possible that the disease is transmitted in this way. Wood has recently expressed the view that sprue is not infrequently mistaken for pellagra in the southern United States.

Trench Fever.—A group of imperfectly classified and ill-understood conditions has been noted in soldiers occupying trenches on the battle line under the designation "trench fever." The onset is quite abrupt, with severe frontal and postorbital headache, followed by rachialgia and pains in the extremities.

The fever rapidly rises to 102°–104°F. and falls rather abruptly to normal about the fourth day. A second rise is frequently noted, so that many of the temperature charts have a saddle back character.

There is no rash and the disease is often diagnosed as influenza, although there are no catarrhal manifestations to justify such diagnosis. The spleen is frequently palpable. The pulse is often slow, thus resembling the group of dengue-like fevers.

Besides the influenza-like type of trench fever another type has been described as the long period one. In this there are frequent relapses, as many as five or six. The fever rise at such times is very short, only lasting a few hours or a day or so. A characteristic pain noted in trench fever is a cutaneous hyperæsthesia over the shins.

Houston and McCloy have thought it possible that the *Enterococcus* is the cause of these fevers. It is considered that lice transmit the disease.

Tsutsugamushi.—A disease of Japan somewhat resembling typhus fever. Supposed to be due to a protozoon transmitted by the Kedani mite.

Verruga Peruana.—A disease of Peru formerly considered as a later stage of Oroya fever.

The eruption of verruga somewhat resembles that of yaws and it was at one time suggested that verruga was simply yaws as influenced by high altitude. Strong and his colleagues found that they could infect rabbits intratesticularly and that lesions resembling those of man could be produced in dogs and monkeys by cutaneous and subcutaneous inoculations. The virus has been transmitted from monkey to monkey. The Wassermann reaction was negative. In extracts from the granulomatous lesions they found a very active hæmolysin. It will be remembered that animals are not susceptible to Oroya fever blood inoculations.

From the fact that it is possible to inoculate a person by rubbing verruga material on a scarified surface it would seem that the infection might be transmitted by insects.

Yellow Fever.—Due to a filterable virus transmitted by the *Stegomyia calopus*. A disease characterized by sudden onset, rachialgia, albuminuria, jaundice and often hæmorrhages about the third day. Pulse becomes slow even with rising temperature. Black vomit often precedes fatal termination. Virus exists in the blood and is filterable.

The virus is present in the blood of the peripheral circulation only during the first three days of the disease. A female *Stegomyia* sucking the human blood during the first three days from onset of fever may become infected but cannot transmit yellow fever to a second person until after the expiration of at least twelve days, during which time some development of the virus, of the character of which we are in ignorance, goes on in the mosquito.

Seidelin has stated that he has found a protozoon, *Paraplasma flavigenum*, in the blood of yellow-fever patients. Authorities generally deny the existence of this parasite.

APPENDIX

A—PREPARATION OF TISSUES FOR EXAMINATION IN MICROSCOPIC SECTIONS

Note. The *most important step in the preparation of sections of tissues for histological examination is proper and immediate fixation.* This step in the technic is often in the hands of the surgeon at the time of the operation or the physician at autopsy and it should be understood that a satisfactory diagnosis can only be made when the pieces of tissue are at *once dropped into a fixative.* Various protozoa, as amœbæ, disintegrate in one or two hours, unless properly fixed and body cells show degeneration after the tissues have been left without fixation for a few hours, which changes may be interpreted as pathological.

Prepare a pint or quart of 5 to 10% formalin solution (2 to 4% formaldehyde) shortly before operation or autopsy. *Drop into the solution* slices of tissue, not more than ¼ inch thick, *as soon as cut.* Leave in the fixative for twenty-four hours or longer when the specimen is to be sent away to a laboratory for diagnosis. *The pathologist will attend to the other steps.*

We use two fixation solutions in routine work, one of 10% formalin and one of Zenker's solution. This latter requires prolonged washing of tissues following fixation and has little advantage over formalin for ordinary purposes.

1. **Fixation:**

a. It is most important that the tissues to be examined be placed in the fixing fluid as soon after death or operation as possible. Degenerative changes are in this way avoided.

b. The piece of tissue to be fixed must not be too large. Using a sharp scalpel, or preferably a razor, a slab of tissue about one-half an inch square and not more than one-fifth of an inch thick should be dropped into the bottle containing the fixative. The bottom of this bottle should have a thin layer of cotton with a piece of filter-paper covering it. There should be at least 20 times as great a volume of fixing fluid as of tissue to be fixed. Delicate tissues, as pieces of gut, should be attached to pieces of glass, wood cardboard, or blotting paper before being placed in the fixative.

c. The most convenient fixative for the average medical man is (1) a 10% solution of ordinary commercial formalin (4% of formic aldehyde gas), either in water or, preferably, in normal salt solution. Fixation is complete in from twelve to twenty-four hours. By placing in the incubator, at 37°C., two to twelve hours in the formalin solution suffices. If fixed in the paraffin oven (56°C.), fixation is accomplished in about one-half hour.

Formalin once used for fixation must be thrown away.

(2) The fixative which probably gives the best histological pictures and with which we obtain the most satisfactory hæmatoxylin staining is Zenker's fluid. This is Müller's fluid containing 5% of corrosive sublimate. It also contains 5% of glacial acetic acid, which latter is only added just before we are ready to fix the piece of tissue. Müller's fluid is:

Pot. bichromate,	2.5 grams.
Sod. sulphate,	1.0 grams.
Water,	100.0 c.c.

Zenker's fluid fixes in about twenty-four hours. After all corrosive sublimate fixatives we should wash the tissues in running water for twelve to twenty-four hours. The precipitate of mercury in the tissues is best gotten rid of by treating the section on the slide with Lugol's solution, rather than the tissue in bulk with iodine alcohol.

(3) In Orth's fluid we add 10% of formalin to Müller's fluid (recommended for nerve tissue).

A saturated corrosive sublimate solution in salt solution with the addition of 5% of glacial acetic acid may be used as a substitute for Zenker's fluid.

(4) Where the tissue is to be examined chiefly for bacteria absolute alcohol is the best fixative. The piece of tissue should be small, not over ⅛ inch thick and suspended by a string to the cork so as not to lie on the bottom where the alcoholic strength tends to become weaker. Better histological details are gotten by fixing for two hours with 80% alcohol and then transferring to absolute for twelve to twenty-four hours.

2. **Dehydration.**—After washing for twelve to twenty-four hours in running water, following corrosive sublimate fixation, or simply washing for a few minutes after formalin, the tissues should be placed in 70% alcohol. They may be kept in this indefinitely. If they are to be sent to a laboratory for sectioning, it is advisable to moisten a pledget of cotton in 70% alcohol and fill in the bottom of the bottle with it. Then drop in the tissues and pack in gently over them sufficient 70% alcohol-saturated cotton to fill up the bottle. All the alcohol should be absorbed by the cotton so that if the bottle should break in transit there would be no damage from the alcohol. The stopper of the bottle should be paraffined or sealed with wax. *Postal regulations forbid alcohol in mail. Send in formalin.*

Tissues may be left in the 70% alcohol twelve to twenty-four hours and should then be transferred to 95% alcohol for an equal time. They are then transferred to absolute alcohol, where they remain from two to twelve hours and are then placed in xylol. The time in xylol should be as short as possible. So soon as the tissue looks clear it should be removed—thirty minutes to two hours.

Bolles Lee is a strong advocate of the superiority of cedar oil over xylol or any other clearing agent for paraffin imbedding. It does not affect delicate structures nor make them brittle even when kept in the cedar oil for weeks or months. Furthermore, it does not matter whether the cedar oil is entirely gotten rid of before sectioning the paraffin as is the case for best results with xylol. Cedar oil will clear from 95% alcohol as well as from absolute alcohol.

3. **Imbedding.**—The tissue is now transferred to melted paraffin. Paraffin melting at 48°C. for winter work, and that melting at 54°C. for summer is to be recommended. The time in the paraffin should not be prolonged. Two hours will ordinarily suffice. Some leave in the paraffin for twelve to twenty-four hours.

Next take a paper box (made of stiff writing-paper folded over a square of wood) and fill with the melted paraffin. As quickly as possible drop in the piece of tissue taken out of the paraffin bath with heated forceps and, so soon as the paraffin begins to solidify on the surface, place the paper box in ice water. When paraffin is rapidly cooled, crystallization is less.

The Acetone Method.—Take the tissues out of the 70% alcohol and place in acetone. After remaining in acetone for one or two hours, the tissues should be transferred to fresh acetone for an equal length of time. Dry calcium chloride in the bottom of the acetone bottles keeps it dehydrated. They should then be placed in xylol for about one-half hour and then embedded in paraffin as directed above.

The Chloroform Method.—The procedure may be the same as in the method of passing through alcohols to xylol, substituting chloroform for xylol and then transferring to paraffin.

Where absolute alcohol is not obtainable, very satisfactory results may be obtained by transferring to a mixture of 95% alcohol and chloroform after immersion in 95% alcohol. Then going from the alcohol-chloroform mixture to pure chloroform, thence to paraffin.

Rapid paraffin imbedding methods.

When a piece of tissue is not more than ¼ inch square and ⅛ inch thick, it is very easy to run it through in three to six hours. Thus:

10% Formalin (in 37°C. incubator),	1 hour.
70% Alcohol (in 37°C. incubator),	1 hour.
95% Alcohol (in 37°C. incubator),	1 hour.
Absolute alcohol (in 37°C. incubator),	½ hour.
Xylol (in 37°C. incubator),	½ hour.
Paraffin (in 55°C. incubator),	½ to 2 hours.

Method of Lubarsch.—In this excellent method small pieces of tissue not more than ⅕ inch thick are placed in a wide test-tube containing 10% formalin for ten to fifteen minutes, changing the fluid twice. Transfer to 95% alcohol ten minutes changing alcohol once. Absolute alcohol, for ten minutes changing twice. Pure aniline oil until tissues are transparent, fifteen to thirty minutes. Xylol, changing two to three times or until the xylol is no longer yellow, ten to twenty minutes. Imbed in paraffin for twenty minutes to one hour. During the entire process keep the test-tube in a water bath or incubator at 50°C. It is necessary to have a good microtome. The best is that of Minot. Very satisfactory sections can be cut with the various types of student microtomes, costing from twelve to twenty dollars.

(In using a hand microtome, a razor with a flat edge is necessary. After experience, sections thin enough for histological but not for bacteriological examination can be made.)

If the piece of tissue is properly dehydrated and imbedded, thin sections (3 to 10μ) should be easily obtained, provided the knife be sharp. One advantage about

the paraffin method is that it is only necessary to have a small part of the blade in proper condition. With celloidin the entire cutting edge must be perfect. Having cut the sections, they should be dropped on the surface of a bowl of warm water (45°C.). This causes the section to flatten out evenly.

Decalcification.—This is best accomplished by fixing in 10% formalin for twenty-four hours, then placing a small piece of the bone (not exceeding ⅓ inch square and ⅛ inch thick) in concentrated sulphurous acid.

This decalcifies in about two to seven days. Wash thoroughly in alkaline water and then in tap water. Pass through alcohols and xylol and imbed and section as before described.

To Stain Sections.—It is first necessary to affix the section to a slide or cover-glass.

To attach the section firmly to the slide, so that it will not become detached in subsequent treatment, pick up a section on a strip of cigarette paper.

A sheet of cigarette paper is cut into about five pieces (½×1½ inch). Inserting the strip of cigarette paper under the section, it is easily lifted up out of the water. Then apply the slip of cigarette paper, section downward, to a perfectly clean slide. Blot with a piece of filter-paper, then strip off the piece of filter-paper leaving the section smoothly applied to the slide. Next place in the 37°C. incubator for twelve to twenty-four hours and the section will be found to be so firmly attached that it will not be dislodged by subsequent treatment.

For Immediate Diagnosis.—Take a very small loopful of albumin fixative (white of fresh egg, 50 c.c., glycerine, 50 c.c.; sodium salicylate, 1 gram) and deposit it on a cover-glass. Now take up a loopful of 30% alcohol (1 drop of 95% alcohol and 2 drops of water) and applying it over the albumin fixative, smear out the mixture uniformly over the cover-glass.

2. Pick up a section on a strip of cigarette paper and apply it to the prepared surface on the cover-glass. Blot with gentle pressure with a piece of filter-paper over the strip of cigarette paper, and strip off this latter, leaving the section attached to the cover-glass.

3. Now, turning the flame of the Bunsen burner down very low or with a small alcohol flame, we hold the cover-glass in a Stewart's forceps, section side up, over the flame and slowly lower it until the paraffin is observed to melt. This shows a temperature of about 50°C. The section is fixed by the coagulation of the albumin at about 70°C. To obtain this temperature lower the cover-glass still more, and the moment vapor is seen to rise from the section it indicates the attachment of the section to the cover-glass.

4. Flood section on cover-glass or slide with xylol; this dissolves out the paraffin. It is better to pour off the first xylol and drop on fresh xylol (one minute).

5. Remove xylol with two applications of absolute alcohol (one minute).

6. Treat specimen with two or three applications of 95% alcohol (one to two minutes).

7. Next wash in water (one to two minutes).

8. Flood specimen with hæmalum or Delafield's hæmatoxylin (three to seven minutes).

9. Wash in tap water for about two to five minutes until a purplish tinge is developed in the section. The alkali in ordinary tap water develops this color.

10. Apply 1 to 1000 eosin (aqueous) for thirty seconds to one minute.

11. Wash in water; then in 95% alcohol; then in absolute alcohol.

12. Apply a few drops of xylol and as soon as the section is perfectly transparent mount in balsam, or immersion oil.

The staining by hæmatoxylin and eosin is the best for the study of the histology of a section. It only requires about ten minutes to run a preparation through for diagnosis by this method.

The reagents are best kept in dropping-bottles.

The staining of sections on slides is exactly as for those on cover-glasses. Coplin's staining jars are very convenient for use in staining slides.

Where the cover-glass method is used, staining by Gram's method, acid-fast staining, capsule staining, etc., may be carried out as for bacterial preparations.

For staining Gram-positive bacteria in sections, the Gram method as for bacterial preparations, using dilute carbol fuchsin or Bismarck brown or safranin as a counterstain, gives good results.

For Gram-negative bacteria stain with thionin as for blood preparations (ten to twenty minutes). Then differentiate in 1 to 500 acetic acid solution for ten to twenty seconds, wash with water, then with 95% alcohol, and quickly through absolute alcohol and xylol.

Naval Medical School Routine Staining of Sections on Slides.—Tissue fixed in formalin.

1. Fix specimen in 10% formalin for 24 hours, then cut into desired blocks $\frac{1}{5}$ inch thick and about $\frac{1}{2}$ inch square.
2. 95% alcohol six to twelve hours. Two changes.
3. Absolute alcohol six to twelve hours. Two changes.
4. Choloroform twelve hours (over night).
5. Paraffin bath two to five hours, 57°C.
6. Imbed—cut 3 to 6 microns thick.
7. Place sections in water warmed to 40°C.
8. Smear thin film of egg albumin on a clean slide.
9. Remove sections from water with a cigarette paper and with section downward blot on the albumin side of the slide with blotting paper.
10. Put slides in 37°C. incubator six to twelve hours.
11. Put slides in 56°C. incubator ten to twenty minutes.
12. Cool.
13. Xylol three to five minutes.
14. Absolute alcohol three to five minutes.
15. 95% alcohol three to five minutes.
16. 70% alcohol three to five minutes.
17. Water three to five minutes.
18. Hæmatoxylin four to eight minutes (Delafield's).
19. Wash in water and decolorize if necessary in acid alcohol.
20. Ammonia water three to five minutes (3 minims to 50 c.c. water).
21. Wash in water.
22. 95% alcohol three to five minutes.
23. Eosin (alcoholic) three to five minutes.
24. 95% alcohol two to five minutes.

25. Absolute alcohol three to five minutes—two changes.

26. Xylol three to five minutes—two changes.

27. Place a small drop of xylol balsam on a cover-glass and place over section on slide.

Nicolle's Method.—1. Stain with Löffler's methylene blue ten to fifteen minutes.

2. Differentiate in 1 to 500 acetic acid ten to twenty seconds.

3. Place in 1% solution of tannin for a few seconds (fixes color).

4. Wash in water, then into 95% alcohol, absolute alcohol, xylol, and balsam.

Weigert's Iron Hæmatoxylin

Solution I

Hæmatoxylin,	1 gram.
Alcohol (95%),	100 c.c.

This must be allowed to ripen for some days and does not keep over six months.

Solution II

Liq. Ferri sesquichlor. sp. gr. 1.124 (about 10%)	4 c.c.
HCl,	1 c.c.
Water,	100 c.c.

Mix equal parts of number one and number two. The mixture only keeps abou three days. The HCl prevents overstaining.

This stain followed by Van Giesen's stain gives more perfect results than any common method of staining. The iron hæmatoxylin intensifies the sharpness of the Van Giesen differentiation.

Other iron hæmatoxylin stains are given under staining for protozoa.

Van Giesen's Stain.—Take of 1% aqueous solution acid fuchsin from 5 to 15 c.c. Saturated aqueous solution picric acid 100 c.c. The method of using is to first stain with hæmatoxylin in the usual way. Then pour on the picric-acid fuchsin solution and allow to stain for one to five minutes. Wash, pass through alcohols and xylol and mount in balsam.

Connective-tissue fibers, axis cylinders, and ganglion cells are stained a bright garnet red. Myelin, muscle fibers, and cells generally are stained yellow. Nuclear staining is that of hæmatoxylin. The stronger stain is used for nerve tissue; the weaker, for demonstrating connective tissue in tumors.

Levaditi's Method.—Take small pieces of tissue, about 2 mm. in thickness, and harden in 10% formalin for twenty-four hours and then in alcohol for the same period; then wash in water for a short period. They are stained in a freshly made solution of silver nitrate 1.5% for three successive days, changing the solution each day, maintaining the blood temperature, and excluding light. The tissue is then placed in a 2% solution of pyrogallic acid, with the addition of 5% formalin. After remaining in this for twenty-four hours, light being excluded, they are passed through 85%, 95%, and absolute alcohol, respectively; embedded in paraffin; and cut in about 5 micron sections. Equally good results may be obtained by allowing the silver nitrate to act at room temperature and embedding in celloidin.

Noguchi has used the following modification in demonstrating spirochætes in

brain and cord: Fix ⅛-inch slabs of tissue in 10% formalin for four or five days. Then place tissues in the following solution: Formalin 10 c.c., pyridin 10 c.c., acetone 25 c.c., absolute alcohol 25 c.c., distilled water 30 c.c. Keep in this solution for five days at room temperature. Then wash in water for one day. Transfer to 95% alcohol for three days and then wash in water for one day. Put tissue in dark bottle in 1½% aqueous solution of silver nitrate for five days at room temperature. Wash in distilled water for five to six hours. Transfer to reducing mixture of 95 c.c. of 4% aqueous solution of pyrogallol and 5 c.c. of formalin. Keep in this solution twenty-four hours. Wash in water and put through alcohol and xylol. Imbed in paraffin.

Romanowsky.—Staining sections with Romanowsky stains is not very satisfactory. The differential staining seems to fade out in passing through the alcohols. This may be avoided by blotting the section after staining and differentiation and then applying the xylol to the blotted section. After staining with Giemsa's stain for ten to fifteen minutes, differentiate with 1 to 500 acetic acid. When the section has a pinkish tinge, wash in water, dry, clear in xylol, and mount.

Good tissue staining may be gotten with Wright's stain. After removing the paraffin with xylol and the xylol with absolute alcohol, pour on a sufficient number of drops of stain and after one minute dilute with an equal number of drops of water. Allow the diluted stain to remain for three to five minutes. Next wash in water, differentiate, until the tissue has a pinkish tinge, in 1 to 500 acetic acid. This differentiation is best done in a tumbler of the dilute acetic acid.

After washing in water, quickly pass through 95% and absolute alcohol, clear in xylol, and mount.

I now use the *panoptic method* for staining tissues. In this I stain with Wright's stain as given above but following the washing of the section we treat this with a dilute Giemsa (1–15) for ten to fifteen minutes. Then wash and differentiate in 1 to 1000 acetic acid in water in a small beaker. When the section assumes a pinkish tinge wash in tap water, then in 95% and absolute alcohol and clear in xylol. Then mount in liquid petrolatum, immersion oil or balsam.

Skin Sectioning.—Of all tissues that of skin offers the greatest difficulty in preparing sections. The best results can probably be obtained by fixation in picro-sublimate (saturated aqueous solution picric acid 1 part; saturated aqueous solution bichloride of mercury one part); to this stock mixture add 5% glacial acetic acid just before using. Fix small pieces of skin six to eighteen hours. Transfer direct to 70% alcohol in which the tissue may be kept indefinitely.

For sectioning run through alcohols to absolute and then to a mixture of absolute alcohol and carbon bisulphide (equal parts). Leave until tissue sinks, then transfer to pure carbon bisulphide until tissue sinks. Then transfer to a saturated solution of paraffin in carbon bisulphide and thence to paraffin. Bisulphide of carbon has the disadvantage of foul odor and inflammability but does not seem to render tissues brittle and difficult to section as does xylol.

Neurological Staining Methods

Neuropathology practically dates from the introduction of Marchi's method of staining in 1885.

Ordinary osmic acid stains both normal and pathological fat. With Marchi's method only the oleic acid of fatty degeneration is stained.

The method is not useful until three or four days have elapsed from the onset of the condition causing the degeneration and it is applicable for only three or four months because by that time phagocytes have taken up the pathological fat which is stained in the Marchi method. The Weigert method is the one to use after a period of three or four months. In Weigert's stain only the normal myelin sheath is stained and the lack of staining of myelin sheaths in degenerated areas is the basis of the stain. For demonstrating axonal reactions or other degenerative changes in nerve-cells, as shown by bulging of the concave sides of the cells, eccentric nucleus and granular appearance of the tigroid bodies, Nissl's method is the best.

For neuroglia fiber staining Mallory's phosphotungstic acid hæmatoxylin is to be recommended.

I. **For Marchi's Method.**—Small pieces of nerve tissue are hardened in Müller's fluid for seven to ten days, and are then transferred to a mixture of two parts Müller's fluid and one part of a 1% osmic acid solution and should remain in this mixture for about seven days. The tissue thus treated is run through alcohols and imbedded in paraffin in the usual way.

II. **For Weigert-Pal Method.**—Thin slices of tissue are fixed in 10% formalin in about four days. The tissue should then be transferred to 5% potassium bichromate for about twelve days. The tissue is then imbedded and sections cut. If only recently mordanted these sections may be at once stained with Weigert's hæmatoxylin for twelve to twenty-four hours (10 c.c. ripened 10% solution hæmatoxylin in absolute alcohol and 90 c.c. water). Wash in water to which about 2% of a saturated solution of lithium carbonate has been added. Now differentiate from one-half to five minutes in ¼% solution of potassium permanganate until the gray matter looks a brownish yellow. Next treat sections with oxalic acid 1 gram, potassium sulphate 1 gram and water 200 c.c. until the gray matter is almost colorless. This takes only a few seconds. Wash in water, pass through alcohols and xylol and mount in balsam.

III. **For Nissl staining** either thionin or Giemsa staining is satisfactory.

IV. For neuroglia fiber staining use **Mallory's Phosphotungstic acid hæmatoxylin.**

Take of hæmatein ammonium,	0.1 gram.
Water,	100.0 c.c.
Phosphotungstic acid crystals (Merck)	2.0 grams.

Dissolve the hæmatein in a little water with the aid of heat, and add it after it is cool to the rest of the solution; no preservative is required. If the solution stains weakly at first, it may be ripened by the addition of 5 c.c. of a ¼% aqueous solution of potassium permanganate, or it may be allowed to stand for a few weeks until it ripens spontaneously.

Making and Staining of Frozen Sections

The various types of ether freezing microtomes are not very satisfactory when only used occasionally. With the general introduction of cylinders containing

compressed carbon dioxide, which is used for aerating waters, we have at hand a practical and convenient method of making frozen sections.

The instrument makers furnish a freezing microtome of the Bardeen type which can be attached directly to the cylinder by a revolving clamp nut.

It is necessary to have a stand to support the iron cylinder in a horizontal position. The tissue, which may be taken at operation for immediate diagnosis, or which preferably has been fixed in formalin for twelve to eighteen hours is immediately placed in water. If the tissues have been in alcohol it will require hours of washing before they can be frozen. The piece of tissue which is to be frozen should not be more than one-fifth of an inch thick. Having placed the piece of tissue on the freezing box of the microtome we turn the valve of the cylinder to allow the gradual escape of gas. When frozen solid, we elevate the freezing box holding the frozen tissue, by revolving a graduated disk with the left hand. In the right hand we firmly grasp a well-sharpened blade of a carpenter's plane mounted in a wooden handle. This is held at an angle of 45 degrees to the polished ways of the microtome. By alternate shoving and withdrawing of the blade, held rigidly, we accumulate on the blade a number of sections. Then dip the blade in a vessel of water to detach the sections which float in the water. Keep repeating the process until numerous satisfactory sections are obtained. Handles for holding the Gillette razor blades are good substitutes for the carpenter's plane.

These sections may be picked up with a strip of cigarette paper and applied to a clean slide upon which a very small loopful of albumin fixative has been smeared out with 30% alcohol. The piece of cigarette paper with the section underneath is then firmly smoothed out upon the slide with filter-paper. The piece of cigarette paper is then carefully stripped off and the section remains attached to the slide. By careful heating over a very small flame, until the vapor just arises, the section is fixed to the slide and we can then stain the section in any way that may be desired. *An excellent and very rapid method* is to drop the piece of tissue just removed at operation or autopsy into a test tube of 10% formalin solution and quickly bring to a boil. Immediately remove the tissue and freeze. The cut sections are separated in the dish of water and picked up on a clean glass slide by manipulation while still in the dish of water. They are floated onto the slide by manipulation with capillary pipettes, the ends of which are sealed off and bent at a right angle. The mounted section is then dried above the flame, the slide being held by the fingers. When dry flood the section with absolute alcohol and add about 6 to 8 drops of thin celloidin (3%). After the celloidin is well mixed with the alcohol pour off excess and plunge slide into hæmatoxylin and proceed as for paraffin sections. Before the final clearing in xylol treat thoroughly with absolute alcohol to remove as much of the excess of celloidin as possible.

NOTE.—The procedures for carrying the tissues through celloidin are not given as it requires perfect condition of the entire cutting surface of the microtome knife and a considerable time for the passage through reagents and celloidin. It is more suitable as a method when sections for class work are to be prepared.

B—MOUNTING AND PRESERVATION OF PATHOLOGICAL SPECIMENS AND ANIMAL PARASITES

To Mount Small Round Worms.—Wash the hook, whip, or filarial worm in salt solution, then drop in 70% alcohol containing 5% of glycerine; the glycerine-alcohol mixture being at a temperature of 60°C. When cool, pour into Petri dishes and allow the alcohol to evaporate in the 37°C. incubator.

Mount in glycerine jelly, preferably in a concave slide, and ring the preparation with gold size. The following is the formula for Kaiser's glycerine jelly: Soak 1 part of gelatin in 6 parts of distilled water for two hours. Then add seven parts of glycerine. To the mixture add 1% of carbolic acid, warm for fifteen minutes, with constant stirring, and then filter through cotton.

To Prepare Tape-worms.—Wash in salt solution. Wrap around a piece of glass as a glass slide and fix in salt solution containing 2 to 5% of formalin. Then keep the preparation permanently in 70% alcohol. If preferred, the specimen may be run through alcohols and xylol and mounted in balsam.

Larvæ.—Mosquito larvæ may either be prepared as for small round worms or they may be dropped into 70% alcohol at 60°C. and then passed through alcohols and cleared in xylol and mounted in balsam. Flukes and insects may require treatment with hot (60° to 70°C.) solution of 10 to 20% sodium hydrate solution. Then wash thoroughly in water and subsequently pass through alcohols to xylol and mount in balsam. Clove oil or cedar oil clears more slowly, but makes specimens less brittle than does xylol. Another satisfactory method is to drop insects or larvæ into acetone at 60°C. and after being in this from one to twelve hours to clear in xylol or clove oil and mount in balsam.

Nematodes.—Looss has a method of first washing a small nematode or delicate fluke in salt solution. Then pouring this first salt solution out of the test-tube in which the washing was carried out, to add fresh salt solution, and then an equal amount of saturated aqueous solution of bichloride of mercury. The shaking is easily carried on in the test-tube. After washing in water the worm is passed through alcohols, one strength of which should contain iodine. Clear in xylol and mount in balsam.

The following method gives very satisfactory results with hookworms:

1. Specimens preserved in 70% alcohol:

(*a*) Place directly in watch glass of carbol-xylol.

(*b*) Observe on stage of dissecting microscope until completely cleared (twelve to twenty-four hours).

(*c*) Mount in chloroform balsam (saturated solution).

2. Specimens preserved in 10% formalin:

(*a*) Dehydrate in absolute alcohol five to fifteen minutes.

NOTE.—Care should be taken not to completely dehydrate the specimen else they will appear shrivelled and distorted.

(*b*) Place directly in watch glass of carbol-xylol.

(*c*) Observe on stage of dissecting microscope until completely cleared (twelve to twenty-four hours).

(*d*) Mount in chloroform balsam (saturated solution).

An excellent method is that of Langeron.

After washing in salt solution fix for a few hours in 5% formalin. Then transfer to lactophenol which has been diluted with an equal amount of water. Allow to remain in this solution for several hours and then transfer to pure lactophenol in which fluid the specimens are to be mounted. Ring with paraffin or with gold size. (To make lactophenol take 2 parts of glycerine and 1 part each of distilled water, crystallized carbolic acid and lactic acid.)

A quick method of preparing small nematodes for examination is to fix them for from two to twelve hours in 5 to 10% formalin, this being heated at 60°C. at the time the worms are dropped into it. Then transfer to the following solution:

Glucose syrup (glucose, 48; water, 52),	100 c.c.
Methyl alcohol,	20 c.c.
Glycerine,	10 c.c.
Camphor, q. s. (a small lump for preservation).	

They may be mounted directly in this and the cover-slip ringed with about 60°C. paraffin, followed with gold size.

Preparations so cleared and mounted in glycerine jelly should also be ringed with paraffin or some cement.

Flukes, cestodes, and nematodes are best stained with carmine. The following is a good formula.

Dissolve, by boiling, 4 grams carmine in 30 drops HCl and 15 c.c. water. Then add 95 c.c. of 85% alcohol and filter while hot. Neutralize with ammonia until precipitate begins to form. Then filter cold.

1. Stain parasites taken from 70% alcohol for five to twenty minutes. 2. Differentiate in 3% hydrochloric acid. 3. Pass through alcohols to xylol and mount in balsam.

Mites, Fleas and Various Small Insects.—By simply taking 1 or 2 drops of liquid petrolatum and mounting the specimen in it then covering with a cover-glass one is able to study the details of these objects almost as well as if they were passed through acetone and xylol into balsam. Liquid petrolatum is also most excellent for mounting the aërial hyphæ of fungi with their sporangia as well as for Romanowsky stained blood smears.

Pathological Specimens.—Pathological tissues which are to be sent to a laboratory for sectioning or to be kept for future study should be fixed by one of the methods given in Section A of the appendix.

Formalin fixation is the more convenient—that with Zenker's fluid the more perfect. After fixation with Zenker's fluid the pieces of tissue must be washed in running water over night.

After fixation the pieces of tissue are transferred to 70% alcohol in which they may be kept indefinitely.

For preservation of gross specimens the method of KAISERLING is generally used. Fix for from one to five days in Solution 1.

Solution 1

Formaldehyde,	200 c.c.
Water,	1000 c.c.
Nitrate of potassium,	15 grams.
Acetate of potassium,	30 grams.

The position of the specimen should be changed from day to day. There must be at least five times as much fluid as specimen. Drain and transfer to 80% alcohol for a few hours, then into 95% alcohol until the natural color is just restored.

Finally preserve in

Acetate of potassium,	200 grams.
Glycerine,	400 c.c.
Water,	2000 c.c.

It is advisable to keep these specimens in the dark as light destroys the natural color.

To Prepare Flies or Mosquitoes for Transmission through the Mails.—Wrap the insect carefully in a piece of tissue paper (toilet-paper answers). Impregnate sawdust with 5% carbolic acid solution and fill around the tissue paper in the box containing them. (Barely moisten.)

It is very satisfactory to take a tube form vial with a cork from the inner surface of which two small shallow holes have been bored, one containing paraformaldehyd, the other camphor. The insect is mounted upon a pin stuck in the cork, which latter is inserted and paraffined externally.

C—PREPARATION OF NORMAL SOLUTIONS

A normal solution is one which contains the hydrogen equivalent of an element, expressed in grams, dissolved in sufficient distilled water to make 1000 c.c. The hydrogen equivalent is the atomic weight of any element divided by its valence. In a base, salt or acid, we use the molecular weight in grams divided by valence.

What may be considered as the valence of a base is shown by the number of hydroxyls combined with it; that of an acid by the number of replaceable hydrogen atoms which it contains.

To make a normal solution, dissolve in distilled water a weight in grams equal to the sum of the atomic weights of the substance divided by its valence, and make up the volume to exactly 1000 c.c.

NaOH is univalent. Na = 23. O = 16. H = 1. Dissolve 40 grams NaOH in water and make up to exactly 1000 c.c.

Oxalic acid is $COOH-COOH+2H_2O$ which gives it a molecular weight of 126. As it contains two carboxyl groups it is dibasic, and it is necessary to divide the molecular weight by 2, so that for a normal solution of oxalic acid we dissolve 63 grams in a volume of distilled water made up to 1000 c.c.

If a chemical laboratory is not accessible one may prepare normal solutions with an error so slight as to be unimportant in clinical work in the following way:

Sodium hydrate being very hygroscopic, it is impossible to accurately prepare a normal solution by directly weighing out the substance. Instead, select perfect crystals of oxalic acid, such as can be obtained in a drug store, and weigh out on the most accurate apothecary scales obtainable exactly 6.3 grams of the most perfect crystals in the bottle. Put these preferably in a volumetric flask and make up with distilled water to 1000 c.c. Less accurate is the use of a measuring cylinder. If care is used this should give N/10 solution of oxalic acid in which the error is less than 1%.

Having N/10 acid at hand, we may prepare N/10 NaOH in the following way: Weigh out an excess of sodium hydrate (5 grams of stick caustic soda) and dissolve in 1100 c.c. of distilled water. Take up 10 c.c. of this solution with a pipette and let it run into a beaker. Add 6 drops of phenolphthalein solution. This gives a violet-pink color. Fill the burette with the N/10 oxalic acid solution and let it run into the sodium hydrate solution in the beaker until the pink is just discharged. Reading off the number of cubic centimeters of the N/10 acid used, we know the strength of the sodium hydrate solution. It is well to repeat the titration and take an average.

If 10.5 c.c. of the oxalic solution were required it would show that the sodium hydrate solution was stronger than N/10, as only 10 c.c. would have been necessary if the NaOH solution had been N/10. It is therefore necessary to dilute the sodium-hydrate solution in the proportion of 10 to 10.5. Measure exactly 1000 c.c. of the too concentrated sodium-hydrate solution and add to it 50 c.c. of distilled water, mix thoroughly, and we have 1050 c.c. of N/10 solution of NaOH. $1000 \times 10.5 = 10{,}500$. $10{,}500 \div 10 = 1050$.

As Acidum hydrochloricum U. S. P. is about two-thirds water (68.1%) to make N/10 HCl, which would require 3.65 in 1000 c.c., it would be necessary to take about three times this amount of U. S. P. acid. Take 12 c.c. of the acid and add distilled water to make 1100 c.c. Put 10 c.c. of this dilute solution in a beaker. Add phenolphthalein solution and titrate. If 11 c.c. of N/10 NaOH were required it would be necessary to add 100 c.c. of water to a volume of 1000 c.c. of the diluted hydrochloric acid. $1000 \times 11 = 11{,}000 \div 10 = 1100$.

Other acid and alkali solutions can be made as for N/10 HCl and N/10 NaOH.

D—CHEMICAL EXAMINATION OF THE BLOOD

Blood Sugar.—Normally we have about 0.1% sugar, or 100 mg. in 100 c.c. of blood. In diabetes we have hyperglycæmia with an increase of blood glucose to twice or even eight times as much. The determination of blood sugar is important in differentiating the so-called "renal diabetes" from true diabetes. In renal diabetes we have a normal blood sugar content. Furthermore, the sugar in the urine rarely exceeds 1% and this glycosuria is not affected by variations in carbohydrate intake as is the case with true diabetes mellitus. Furthermore, there are no symptoms, such as thirst, excessive polyuria, loss of weight, etc.

The micro-method of Bang is the one used by many workers in making this determination. Objections to it that we have noted have been the necessity for an accurate chemical balance for the weighing of the drop or two of blood which is taken up on a previously weighed piece of filter-paper. Again the titration of the cuprous chloride with N/200 iodine solution has to be carried out with exclusion of air and furthermore, the previous boiling gives changing results according to time of process. In my opinion it can only be carried out accurately in the hands of an experienced chemical worker.

Myers and Fine Modification of Lewis and Benedict Method.—For taking blood, whether for sugar or nonprotein nitrogen determination, we use the blood system described under "Blood Examination." With a graduated centrifuge tube we make a blue pencil mark at 2 c.c. for sugar determinations, or at 5 c.c. for nonprotein nitrogen ones.

A small pinch of finely powdered potassium oxalate is dusted into the bottom of the graduated centrifuge tube. As the blood drops into the tube we keep agitating the tube so that the oxalate dissolves and prevents coagulation of the blood. Instead of the powdered potassium oxalate we may put 1 c.c. of a 2% solution of the oxalate in the centrifuge tube and make a mark for the blood at the 3 c.c. or 6 c.c line. Folin uses a 2 or 5 c.c. pipette into which potassium oxalate has been dusted and which is connected with the needle in the vein.

Transfer the 2 c.c. of oxalated blood, to which has been added exactly 8 c.c. of water, to a test-tube. Then add 0.2 gram of picric acid. Mix thoroughly and after standing five minutes filter through small dry filter. Three c.c. of filtrate are placed in a tall test-tube and 1 c.c. of 20% Na_2CO_3 added. Tube is placed in boiling water bath for fifteen to thirty minutes. Now cool and make volume up to 20 c.c. and compare by Duboscq colorimeter with a known dextrose solution (0.1%) similarly treated.

Instead of the Duboscq or Hellige colorimeter one can use the following method which is sufficiently accurate for clinical purposes.

In a case of diabetes make up a 0.2% sugar solution instead of the 0.1% and treat as above with the picric acid. Then make up accurately to 20 c.c. in a graduated cylinder. Now with the 20 c.c. of yellow-colored solution from the blood in a tube between the thumb and forefinger of the left hand we add to the empty tube alongside the known 0.2% solution from the graduated cylinder. When the colors match we read off the amount used from the cylinder and calculate the strength of the blood sugar tube. If 15 c.c. were required it would show that the sugar of the blood equalled 0.15%.

Blood Sugar Estimation According to Epstein using Kuttner's Micro-colorimeter.—The same calibrated and color tube employed in the Sahli-Gower hemoglobinometer has been adopted for this instrument.

The instrument consisting of a closed upright box (dimensions 2.5 cm. by 8.5 cm.) is smaller than the Sahli. At the top there are two openings: one for the color tube, the other for the calibrated tube. Near the lower front part is a window provided with "A", a blending effect prism (A—Helmholtz double plates), which serves the purpose of having the colors of both tubes appear close together, forming one continuous color band. The prism is easily removed for cleansing and can be quickly readjusted. A sliding door (B), which can be raised and lowered, protects the prism from dust and injury. The color standard and calibrated tube are separated from each other by a partition, which prevents light from being reflected from one tube to another.

According to Dr. Epstein's modification of Benedict's method, the procedure is as follows:

0.2 c.c. of blood is drawn with pipette No. 3 and discharged into a graduated test tube containing 1 drop of 2% fluoride of sodium solution or potass. oxalate, the pipette rinsed two or three times with water, the rinsings added to the test-tube, then water to the 1 c.c. mark. Saturated solution of picric acid is added up to the 2.5 c.c. mark, the mixture well shaken and now either filtered or centrifuged. Of the clear, supernatant liquid 1 c.c. is measured with a pipette and boiled down to 2 or 3 drops in a boiling test-tube, 0.5 c.c. of a 10% sodium carbonate solution added and again boiled to crystallization, which occurs when the contents are concentrated

to about 2 to 3 drops and becomes brownish-red in color, according to the amount of sugar present, when the process is completed. A few drops of water are added, the tube warmed and contents transferred to the calibrated tube of the Micro-colorimeter. The tube is rinsed with sufficient water, a few drops at a time, the total volume reaching the mark 50 and compared in color with the standards. If it is darker than A, but lighter than B, then the former is used as standard, and compared in the instrument by adding water drop by drop to the fluid and mixing. When the colors have exactly matched, the height to which the fluid has risen is read off on the scale, this figure divided by 1000 representing the percentage of sugar in the blood. If the darker tube B is used, the result must be multiplied by 2. Using this tube with the meniscus having reached mark 85 on the scale, then $85 \times 2 = 170$.

$$170 \div 1000 = 0.17\%$$

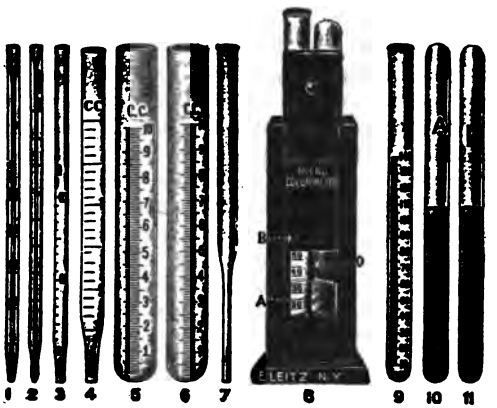


FIG. 140.—Kuttner s Micro-colorimeter.

As it often happens that diabetic blood may contain 0.3% or more of sugar, it would be necessary to use less blood. It is better to take 2 specimens, one as already stated and another with pipette No. 2, which measures only 0.1 c.c. of blood, then proceeding in the same manner as already described, but multiplying all obtained figures again by 2. Computation would then be as follows: If the color matched the darker tube B at 85, but using only 0.1 c.c. of blood then

$$85 \times 2 \times 2 = 340$$
$$340 \div 1000 = 0.34\%$$

If the color matched the darker tube B at 140, the computation is thus:

$$140 \times 2 \times 2 = 560$$
$$560 \div 1000 = 0.56\%$$

Normal blood contains from 0.06% to 0.12% sugar. It is best to take the blood before breakfast.

Blood Creatinin.—From 0.5 to 2 c.c. of blood or serum may be taken for this test. A convenient amount to work with is 1.5 c.c. The required amount is measured with a pipette into a short wide test-tube suitable for boiling, the pipette rinsed several times with water and the rinsings added. It is then further diluted with water and reagents so that every c.c. of the dilution contains 0.1 c.c. of serum or blood. It is diluted at first with about five volumes of water, one drop of a 0.1% acetic acid solution (1) and 2 drops of a sodium acetate solution (1/10 saturated), (2) are added for every 0.1 c.c. of serum used. This is boiled and the tube with contents is rapidly cooled by holding it in running tap water. The cooled mixture is made up to volume so that each c.c. of the mixture represents 0.1 c.c. of blood or serum. A few drops of colloidal iron may be added to the water used for making up to volume which assists in clarifying. This is then filtered. Ten c.c. of the water clear filtrate (10) representing 1 c.c. of blood or serum is boiled in a test-tube nearly to dryness and cooled. Now 0.3 c.c. of saturated picric acid solution, 0.1 c.c. of 10% sodium hydroxide, and 0.2 c.c. of water are added. The test-tube is rotated in such manner that the reagents come in contact with all of the creatinin and allowed to stand six minutes for the color to develop. After this lapse of time, it is poured into the calibrated tube (9) and rinsed with water, using a few drops at a time until it has reached the 50 mark on the scale with added rinsings. It is now ready to be compared to the standards. If it is lighter than the standard, No. 2, then the standard No. 1 must be used for the comparison. Water is added drop by drop, until the colors match. The height to which the fluid has risen on the scale is noted.

The method of calculation is simple. Each division on the scale represents $\frac{1}{100}$ mgm. per 100 c.c. of blood. For example, the color having matched at 90, this will be equivalent to $\frac{9}{100}$ or 0.9 mgm. per 100 c.c. When the standard No. 2 is used, the result is multiplied by 2. For example, the color matches the Standard No. 2 when the reading is 140, then

$$\frac{140}{100} \times 2 = 2.8 \text{ mgms. per 100 c.c. blood}$$

In the event of the color being still darker, then the original quantity should be diluted to 2, 3 or more c.c. with water and a sufficient amount poured into the calibrated tube up to the 50 mark for the estimation, but the result must again be multiplied by 2, 3 or more, as the case may demand.

The amount of creatinin usually present in 100 c.c. of normal blood is about 1 mgm.

NOTE.—The picric acid used in the above processes should not turn brownish red within thirty minutes after the addition of NaOH. Should such a color develop another and suitable sample of picric acid should be obtained.

Nonprotein Nitrogen of Blood.—Normally we have from 25 to 35 mg. of nonprotein N in 100 c.c. of blood. This is greatly increased in chronic nephritis and especially in uræmia. The nonprotein N is more easily and accurately determined than the urea N. Of course, the urea N makes up about one-half of the normal nonprotein N and in uræmic conditions it may constitute from 80 to 90% of the nonprotein N. Hawk gives urea N as 12 to 23 mg. per 100 c.c. of blood. In uræmia it may amount to 70 to 300 mg. with a nonprotein N increase of from 90 to 350 mg. per 100 c.c. of blood.

To Estimate Nonprotein N.—To 5 c.c. of the oxalated blood add 75 c.c. of absolute alcohol, mix well and let stand for an hour. Filter through small (9 cm.) dry filter, collecting as much filtrate as possible (the filtrate should be collected in a cylinder to prevent evaporation). To the filtrate add about 5 drops of a saturated alcoholic solution of zinc chloride, let stand another hour and again filter. Measure off 64 c.c. of this filtrate (this represents 4 c.c. of blood). In a 200×20 mm. test-tube place enough filtrate to give a depth of fluid of not more than one inch and evaporate to dryness on water bath; again place a similar portion of the filtrate in the tube and evaporate. Continue this method of evaporation until the full 64 c.c. of filtrate is disposed of. To the residue in the tube add 1.5 c.c. of concentrated sulphuric acid, 1 drop of dilute (1%) copper sulphate solution and 1 gram of potassium sulphate and heat over a very small flame, keeping the solution boiling briskly, until the solution, which at first blackens, becomes clear and colorless. Cool, add about 10 c.c. of water, agitate until solution is complete, add 5 or 6 drops of solution of phenolphthalein, cautiously add 10% NaOH until the solution is nearly neutralized, add 2 grams of potassium oxalate, stir until dissolved, then add very dilute NaOH until the soultion assumes a very faint pink shade, then add from 5 to 10 c.c. of neutral 20% formaldehyde (50% formalin). From a burette now add N/50 NaOH until faint pink color is produced. The number of c.c. of N/50 NaOH required×0.28=number of milligrams of nonprotein N in 4 c.c. of blood.

Urea of Blood.—To 5 c.c of oxalated blood in a test-tube add 25 milligrams (1 tablet) of Urease-Dunning, the tablet having been previously crushed and suspended in 2 or 3 c.c. of water. This mixture is placed in a water-bath at 35° to 40°C. for two hours in order to convert the urea into ammonium carbonate. Then transfer with sufficient washings to the second cylinder jar of an aerating apparatus as shown in No. 1, Fig. 141, add about 1 gram of sodium carbonate, alcohol equal in volume to the blood and washings and about 2 c.c. of kerosene oil to prevent foaming. The aërating apparatus is immediately connected. The first cylinder contains about 25 c.c. of dilute sulphuric acid, the second cylinder contains the mixture of blood, while the third cylinder contains 25 c.c. of N/50 hydrochloric acid to which has been added about 2 c.c. of toluene to prevent foaming. A rapid current of air is drawn through the apparatus by means of a filter suction pump for one hour in order to transfer the ammonia from the blood to the hydrochloric acid. After the aëration is completed the excess of acid is titrated with N/50 sodium hydroxide using alizarin sulphonate or methyl orange as an indicator. Each cubic centimeter of the N/50 hydrochloric acid that has been neutralized by the ammonia formed from the urea is equal to 0.6 milligrams of urea.

Hydrogen-ion Concentration of the Blood.—Levy, Rowntree and Marriott have proposed this determination as an index of the reaction of the blood. The test may be made either on oxalated blood or with serum. The hydrogen-ion concentration of the serum of normal persons varies from p H 7.6 to p H 7.8, that of oxalated blood from p H 7.4 to p H 7.6. The exact point of neutrality, p H 7, is only reached in severe uncompensated acidosis and a reaction of p H 8 is only possibly obtainable after administration of alkalis.

One to 3 c.c. of clear serum or of blood is run, by means of a blunt-pointed pipette, into a dialyzing sac which has been washed inside and outside with salt solution and which has been tested for leaks by filling with the salt solution. The

sac is lowered into a small test-tube (100×10 mm., inside measurements) containing 3 c.c. of the salt solution, until the fluid on the outside of the sac is as high as on the inside. From five to ten minutes are allowed for dialysis. The collodion sac is

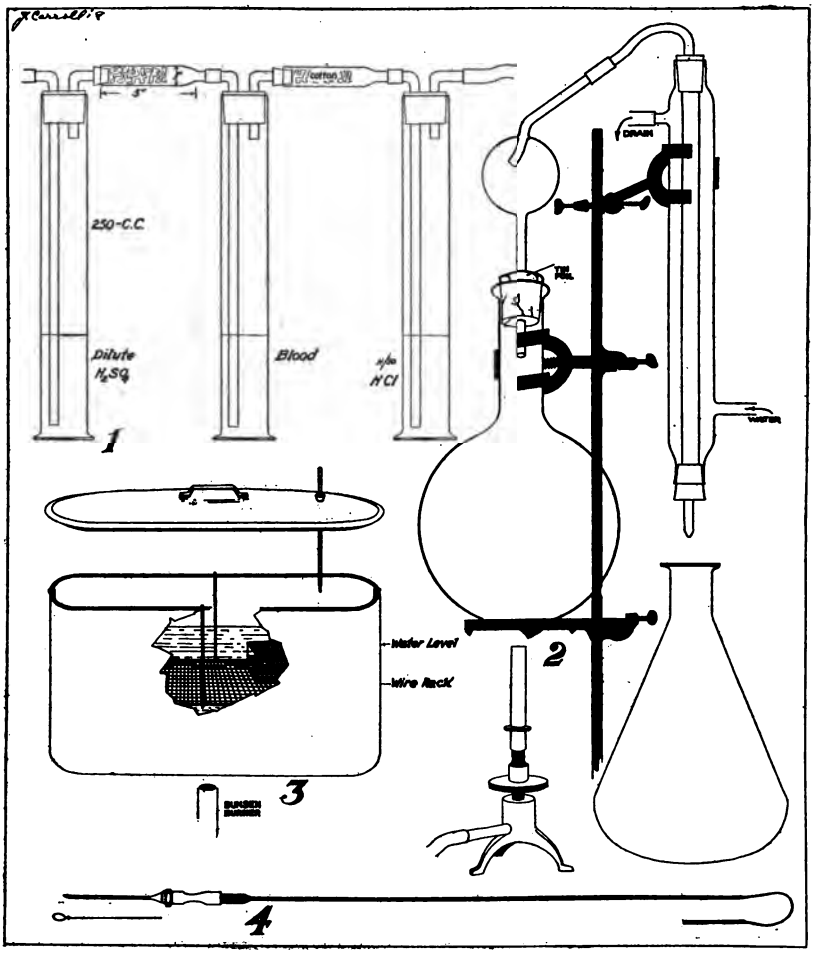


FIG. 141.—1. Apparatus to be connected with filter pump for blood urea determination. 2. Apparatus for distillation in making colloidal gold solution. 3. Substitute for expensive Wassermann water bath. 4. Capillary tube attached to needle for taking blood from vein in coagulation test.

removed and 5 drops of the indicator (an aqueous 0.01% solution of phenolsulphonaphthalin) are thoroughly mixed with the dialysate. The tube is then com-

pared with the series of standards until the corresponding color is found, which indicates the hydrogen-ion concentration present in the dialysate.

These tests may be carried out with 3 c.c. of blood or serum. The same results are obtained with 1 c.c. of blood or serum on the inside of the sac, and with this amount it is immaterial whether there is 1 or 3 c.c. of salt solution on the outside.

For the comparsion of tubes with standards a good light (natural or artificial) and a white background are requisites. Readings must be made immediately. The tube matching most closely is selected and also the tubes on either side of it. These are critically inspected against a white background. Changing the order of the tubes often makes differences more apparent.

Hynson, Westcott and Co. prepare a set of standards with the hydrogen-ion concentration marked on each ampule. The dialyzing collodion sacs are made as for the Abderhalden test or they may be purchased.

E—CHEMICAL EXAMINATION OF URINE

For the prevention of decomposition when a urine is not examined shortly after voiding, chloroform (10 to 20 drops added to a tightly corked bottle) or formalin (4 or 5 drops to a pint of urine) are ordinarily employed. Formalin is better for microscopical material, but, owing to its reducing power, should be substituted by boric acid in urine to be examined for sugar. For clearing urine, turbid by reason of bacteria, rubbing up with Talcum purificat., U. S. P., and filtering is recommended. *It is advisable to call for a fresh specimen and not to examine a deoomposed urine either for casts or albumin.*

A twenty-four-hour specimen is necessary for accurate work. The urine should be collected in clean separate bottles. Where pus comes from the bladder the proportion of pus in each bottle will be practically the same; if from the kidneys the amount will vary in the different bottles.

The amount of urine varies in different individuals (water or beer habit). It is usually given as from 1000 to 1500 c.c.

Long proposes to substitute 2.6 for Hæser's coefficient which, if multiplied by the two final figures of the specific gravity taken at 25°C., gives the weight of urinary solids in 1000 c.c.

Albumin.—Practically serum albumin alone is clinically important.

The two usual tests are 1. Heat test and 2. Heller's nitric acid test. For the former, add 3 to 10 drops of 5% acetic acid to the perfectly clear urine in a test-tube and bring to a boil. By boiling the upper portion a turbidity in contrast with the clear lower portion may be obtained. I usually prefer to heat the urine and after the boiling add the 5% acetic acid, drop by drop. This will clear up the turbidity due to phosphates.

A more delicate test for albumin is the following: Add to a test-tube half filled with filtered urine one-fifth its volume of a saturated aqueous solution of sodium chloride; heat to the boiling-point; add 2 to 5 drops of 50% acetic acid and heat again. This test may serve to distinguish nucleo-albumin, as most forms of nucleo-proteid found in urine do not react to the test, while serum albumin does. Thus where a positive nitric acid test is present, and no precipitate occurs with this test, the proteid present is usually nucleo-proteid.

For Heller's test, pour a small amount of nitric acid into a narrow test-tube and, while holding the tube at an angle of about 45 degrees, superimpose a layer of the urine to be tested, which is delivered drop by drop from a pipette and allowed to flow down the side of the tube.

This test can be converted into a quantitative one which is sufficiently accurate for clinical purposes. It is based on the fact that a specimen of urine containing 0.003% of albumin will give a perceptible ring at the layering of the urine and acid in two minutes. If the ring appears at once or in a few seconds the albumin content is greater. From the qualitative test an idea can be formed as to the amount of albumin which the urine contains, a heavy ring forming immediately showing a considerable albumin content. Probably the highest elimination of albumin is found in chronic parenchymatous nephritis where it may run from 1 to 3%. In an ordinary case of acute nephritis 0.5% would be an average content.

Recently I have been using for both qualitative and quantitative albumin tests the apparatus shown in Fig. 9. This is simply a 5-inch piece of ¼-inch soft glass tubing heated at a point 2 inches from one end, drawn out about 2 inches and bent to form a U tube with one end shorter than the other. This form of tube enables one to perform two tests with the same column of nitric acid and is easily cleaned and dried. They may be kept suspended around a glass tumbler's rim. Taking up a small amount of nitric acid with a capillary bulb pipette it is deposited in the capillary curve of the bent tube. This acid pipette should be kept attached to the acid bottle. With a second pipette the urine is deposited in the short arm of the U tube and the presence of albumin shows by a distinct ring at the junction of urine and acid in the clear capillary tubing. The long arm will serve for the introduction of a different specimen of urine for the albumin test.

For quantitative test we dilute the filtered urine with one or more parts of normal salt solution according to the intensity of the albumin ring. A very convenient way of making the dilution is with a graduated centrifuge tube. Make a 1 to 10 dilution of the urine, mix and draw up with a bulb pipette and deposit in the short arm of the U tube containing nitric acid. A distinct ring forms in two to three seconds. Pour off one-half of the diluted urine and make up with an equal amount of saline. Deposit this 1 to 20 dilution in the long arm. The ring forms in about a minute. With further testing it is found that a 1 to 40 dilution shows a perceptible ring in just two minutes. This final and successful dilution multiplied by 0.0033 gives the percentage of albumin in the urine ($40 \times 0.0033 = 0.13\%$).

Should it be desired to determine the nature of the proteids present either in urine or in exudates or transudates the following method is applicable. Determine the percentage of total proteid by the method employed above. Then throw down the globulins by the addition of an equal amount of a saturated solution of ammonium sulphate, filter and estimate the proteid content of the filtrate. The difference between that and the total gives the percentage of globulin. The filtrate is now treated with 5% acetic acid until a precipitate of nucleo-proteid ceases to form; the fluid is filtered and the clear filtrate (which should not show any turbidity with a drop of 5% acetic acid) is tested for its proteid content, which represents the serum albumin. When the combined percentage of globulins and serum albumin is subtracted from the total proteid percentage we have the percentage of nucleo-proteid.

Esbachs' Quantitative Method for Albumin.—The use of the Esbach tube is attended with some uncertainty, whether using the original Esbach solution or that devised by Tsuchiya, the precipitate at times refusing to settle. The method of using the tube is to add the urine to the U mark and then the reagent to the R mark, mix, and allow to stand in upright position. If Esbach's reagent has been used the reading is made at the end of twenty-four hours, but when Tsuchiya's reagent is employed the reading is made at the end of two hours. The number at the end of the line which corresponds to the upper limit of precipitate will be the number of grams of dry albumin per liter of urine. Esbach's reagent consists of 10 grams of picric and 20 grams of citric acid dissolved in 1 liter of water. Tsuchiya's reagent is made by dissolving 1.5 grams of phosphotungstic acid in a mixture of 5 c.c. strong hydrochloric acid and 95 c.c. of 95% alcohol. In this method also dilution must be resorted to if albumin or specific gravity are excessive.

Nucleo-protein.—Increased quantities of this protein occur in pyelitis, nephritis, and inflammations of the bladder.

For its detection albumin, if present in any considerable quantity, must be removed by boiling as described above. Then place 10 c.c. of this urine in a small beaker, dilute with 3 volumes of water and make the reaction very strongly acid with acetic acid. If the solution becomes turbid it is an indication that nucleo-protein is present.

Bence-Jones Body.—(Albumose.) Perform the heat test for albumin. The appearance of a heavy precipitate which partially clears on boiling suggests albumose. If albumose is present a cloud will appear in the filtrate on cooling. The precipitate formed with nitric acid, if due to albumose, disappears with heat, that of serum albumin does not.

As another test for the *Bence-Jones body*, usually present in multiple myelomata, that of Boston is of value. Mix 15 c.c. urine in a test-tube with an equal amount of saturated NaCl solution. Add 2 c.c. of 40% NaOH solution and shake the contents of the tube thoroughly. Heat the upper contents of the tube to boiling and add lead acetate solution (10%) drop by drop continuing the heating. A brown to black precipitate (sulphur) shows this form of albumin.

In tests requiring the removal of albumin boil the urine and add dilute acetic acid until the precipitate is flocculent, then filter.

Sugar

Fehling.—Pour equal parts of Fehling's copper solution (34.639 grams of copper sulphate in 500 c.c. of water) and Fehling's alkali solution (173 grams sodium potassium tartrate and 50 grams sodium hydrate in 500 c.c. water) into a test-tube. Mix and dilute the deep blue solution with 2 parts of water. Heat the upper portion of the diluted Fehling's solution in the flame to boiling and drop in from a pipette the urine to be examined. A yellowish to red precipitate shows the presence of sugar.

Fehling's test will show the presence of $\frac{1}{100}$ of 1% of glucose in an aqueous solution but is vastly less delicate for sugar in urine. This is due to the power of the creatinin in urine of holding the reduced suboxide of copper in solution.

An important point is that the creatinin is broken up by prolonged boiling hence the puzzling precipitates one gets at times after a long period of boiling are explained in this way. Glycuronic acid may cause a doubtful reaction. If the precipitated cuprous oxide is in very fine granules the color is greenish, if less fine, greenish yellow and if quite coarse, reddish.

Creatinin holds in solution the copper suboxide formed by uric acid as well as that resulting from very small glucose content of urine.

As a test for doubtful glycosuria it is well to give 100 grams of pure glucose. A normal person should deal with such an amount without showing sugar reaction of the urine.

Phenylhydrazin (Kowarsky).—Mix 5 drops of pure phenylhydrazin in a test-tube with 10 drops of glacial acetic acid. Shake lightly and add 15 drops of saturated solution of NaCl. This makes a pasty mixture. Now add 10 c.c. of the urine and bring carefully to a boil over a small flame and continue to boil gently for two minutes. Upon cooling a yellowish crystalline precipitate falls more or less rapidly according to the sugar content of the urine. If the urine contains 0.2% or more of sugar the precipitate appears in a few minutes. The test is sensitive for 0.03% of sugar.

Fermentation Test.—This is the surest test for sugar in the urine. It will show the presence of 0.05% of glucose. Instead of the Einhorn apparatus one may be extemporized by taking a 50 c.c. cylinder, filling it to overflowing with the urine which has previously been rubbed up with a piece of compressed yeast the size of a hazelnut. The urine should be made acid with tartaric acid to prevent ammoniacal decomposition with the formation of CO_2. A small 3-inch test-tube is filled with the yeast-treated urine and dropped mouth downward into the 50 c.c. cylinder. The apparatus is incubated for twenty-four hours and the presence of gas in the closed end of the test-tube shows that sugar was present. A control to determine that the yeast does not contain sugar is advisable. To utilize this test as a quantitive one, first accurately take the specific gravity of the urine; then add the yeast and fill the test-tube and cylinder as directed above. Next pour off or pipette off the urine exactly to the 50 c.c. mark. Incubate for twenty-four to forty-eight hours and make up the loss by evaporation, with distilled water. After the urine has cooled down to room temperature the contents of tube and cylinder are thoroughly mixed (the small tube having been withdrawn with a pair of forceps), then filtered to remove the sediment of yeast and then brought to the exact original volume of 50 c.c. with distilled water to make up the loss by evaporation. (If there should be doubt as to the completion of the fermentation of the glucose a qualitative test for sugar can be made.) The specific gravity is again taken and the difference between this and the first reading multiplied by 0.23. Example: Specific gravity of unfermented urine, 1.030, that of urine after incubation, 1.022. Difference, $8 \times 0.23 = 1.84\%$.

It is advisable to have two good urinometers, one to register from 1000 to 1025, a second to register from 1025 to 1050.

Benedict's New Method for Quantitative Determination of Sugar in Urine

The solution for quantitative work has the following composition:

Copper sulphate (pure crystallized)	18.0 gm.
Sodium carbonate—crystallized (100 grams of anhydrous salt will answer)	200.0 gm.
Sodium or potassium citrate	200.0 gm.
Potassium sulphocyanate	125.0 gm.
5% potassium ferrocyanid solution	5.0 c.c.
Distilled water to make total volume of	1000.0 c.c.

With the aid of heat dissolve the carbonate, citrate and sulphocyanate in enough water to make about 800 c.c. of the mixture, and filter if necessary. Dissolve the copper sulphate separately in about 100 c.c. of water and pour the solution slowly into the other liquid, with constant stirring. Add the ferrocyanid solution, cool and dilute to exactly 1 liter. Of the various constituents, the copper salt only need be weighed with exactness. Twenty-five c.c. of the reagent are reduced by 50 mg. of glucose.

Sugar estimations are conducted as follows: The urine, 10 c.c. of which should be diluted with water to 100 c.c. (unless the sugar content is believed to be low), is poured into a 50 c.c. burette up to the zero mark. Twenty-five c.c. of the reagent are measured with a pipette into a porcelain evaporating dish (25–30 cm. in diameter), 10 to 20 grams of crystallized sodium carbonate (or one-half the weight of the anhydrous salt) are added, together with a small quantity of powdered pumice-stone or talcum, and the mixture heated to boiling over a free flame until the carbonate has entirely dissolved. The diluted urine is now run in from the burette, rather rapidly until a chalk white precipitate forms, and the blue color of the mixture begins to lessen perceptibly, after which the solution from the burette must be run in a few drops at a time, until the disappearance of the last trace of blue color, which marks the end point. The solution must be kept vigorously boiling throughout the entire titration. If the mixture becomes too concentrated during the process, water may be added from time to time to replace the volume lost by evaporation. The calculation of the percentage of sugar in the original sample of urine is very simple. The 25 c.c. of copper solution are reduced by exactly 50 mg. of glucose. Therefore the volume run out of the burette to effect the reduction contained 50 mg. of the sugar. When the urine is diluted 1:10, as in the usual titration of diabetic urines, the formula for calculating the per cent. of sugar is the following:

$\frac{0.050}{X}$ times 1000 equals per cent. in original sample, wherein X is the number of cubic centimeters of the diluted urine required to reduce 25 c.c. of the copper solution.

In the use of this method chloroform must not be present during the titration. If used as a preservative in the urine it may be removed by boiling a sample for a few minutes, and then diluting to its original volume.

This solution will keep indefinitely and it is claimed by Benedict, that comparison with the polariscope and by Allihn's gravimetric process will show it to be more accurate than any of the ordinarily used methods.

APPROXIMATE QUANTITATIVE ESTIMATION with Fehling's solution. (One c.c. of Fehling's solution is reduced by 5 mg. glucose.)

Measure off 2 c.c. of Fehling's solution in a pipette and put in a test-tube or small beaker and dilute with 20 c.c. of water.

Bring the diluted Fehling's to boiling and drop in drop by drop the urine from a dropping-bottle for which the number of drops per cubic centimeter has been noted. Estimating 20 drops to the cubic centimeter if 2 drops of urine are required to reduce the copper it would show a sugar percentage of the urine of 10. Four drops 5%, 8 drops 2.5%, 16 drops 1.25%, 32 drops 0.6%, 64 drops 0.3%, 100 drops 0.2%.

Nylander's Bismuth Reduction Test.—Put 5 c.c. of urine in a test-tube and add 0.5 c.c. Nylander's reagent, then heat for five minutes in a boiling water-bath. If sugar be present the mixture will darken to become black on standing. For the reagent digest 2 grams of bismuth subnitrate and 4 grams Rochelle salts in 100 c.c. of 10% KOH solution. Cool and filter.

Urinary Tests in Connection with Acidosis

The determination of the ammonia quotient, which is the ratio of N eliminated as ammonia to total nitrogen elimination, has assumed great importance by reason of its connection with various forms of acid intoxication, as in diabetes, pernicious vomiting of pregnancy, and various hepatic diseases.

The degree of acidosis is better determined by the quantitative estimation of nitrogen elimination as ammonia than by estimating quantitatively the amount of diacetic and β-oxybutric acid in the urine. Normally we have about 0.7 gram of ammonia eliminated daily. In acidosis this may rise to 5 or 10 grams and instead of being from 3 to 5% of the total N, it may amount to 30 to 50%.

In the acidosis connected with chronic nephritis it has been found that the preformed ammonia is often below normal, indicating a defect in the normal neutralizing action of ammonia. Where there is excessive formation of acid bodies as in diabetes, or when liver cell degeneration interferes with the normal conversion of ammonia into urea we have an increase in urinary ammonia output.

Reaction.—Urine may be quite acid in acidosis. The reaction of the mixed twenty-four-hour excretions is acid to litmus. At times during this period, especially after meals, the reaction may be alkaline. It is not positively known to what the acidity is due, some authorities considering it due to sodium dihydrogen phosphate, while others attribute it to organic acids. The acidity being very largely due to food, naturally it would vary in health, as the nature of food varies. The degree of acidity under normal conditions is such that from 40 to 60 c.c. of N/1 alkali will be required to neutralize the twenty-four-hour excretion.

The acidity can be determined by measuring 25 c.c. of sample into beaker, adding 75 c.c. of distilled water, and also, if available, 10 grams of neutral potassium oxalate and finally 3 or 4 drops of phenolphthalein. From a burette add N/10 sodium hydrate until a permanent faint red or pink color is obtained. High-colored urines must be diluted with more than 75 c.c. of water before the end reaction can readily be detected. Normally, 6 to 8 c.c. neutralizes 25 c.c. urine.

Formalin Methods for the Estimation of Ammonia

Free ammonia reacts with formalin to form hæxamethylenetetramine. If sodium hydrate is added to neutralized urine in the presence of formalin free ammonia is liberated and reacts with the formalin. So soon as all the ammonia has been liberated, the end reaction occurs.

Ronchese first utilized this principle and Mathison found that potassium oxalate made the end reaction sharper. Brown found that preliminary clearing with lead subacetate made the end reaction still sharper and removed certain nitrogenous substances which reacted with formalin making the result only about 5% higher than with Schaffer's method. The technic is as follows: About 60 c.c. of filtered urine are treated with 3 grams of basic lead acetate, well stirred, allowed to stand a few minutes and filtered. The filtrate is treated with 2 grams of neutral potassium oxalate well stirred and filtered; 10 c.c. of the clear filtrate are diluted to 50 c.c. with distilled water; a few drops of 1% phenolphthalein solution are added. The mixture will be slightly alkaline or acid. Five grams potassium oxalate are added and stirred. It is exactly neutralized with decinormal NaOH or H_2SO_4. Twenty c.c. of 20% commercial formalin, previously made neutral, are added, and the solution again titrated with decinormal NaOH to neutralization. Every cubic centimeter of decinormal NaOH corresponds to 0.0017 gram NH_3. The quantity of ammonia is then calculated on the basis of the twenty-four-hour volume. Example: The 10 c.c. of urine required 4 c.c. N/10 NaOH to give a pink color. $4 \times 0.0017 = 0.0068$. Then 100 c.c. urine would contain 0.068 and 1000 c.c. (twenty-four-hour urine amount) 0.68 gram of ammonia.

Estimation of Total Nitrogen

Principle.—The nitrogenous material of the urine is converted into ammonium sulphate on boiling with H_2SO_4. The ammonia is then estimated as described under estimation of ammonia by the formalin method.

Technic.—Solutions required:

1. Twenty per cent. commercial formalin previously made neutral with NaOH.
2. N/10 NaOH.
3. Forty per cent. NaOH.

Ten c.c. of filtered urine are pipetted into a Kjeldahl or Koch flask; 10 c.c. of concentrated H_2SO_4 and 10 grams K_2SO_4 are added. The mixture is heated over a free flame, gently at first to avoid foaming, and is finally brought to a boil, which is continued until the mixture is perfectly clear, usually requiring forty-five minutes to an hour. The contents are cooled and quantitatively transferred to a 200 c.c. volumetric flask and 1 c.c. of phenolphthalein solution added. The greater part of the acidity is now neutralized by adding about 30 c.c. of the 40% NaOH. It is cooled under a water tap and made up to the 200 c.c. mark; 10 c.c. are taken, diluted in 50 c.c. with distilled water and exactly neutralized with N/10 NaOH. Twenty c.c. of the formalin solution are now added and the titration again performed. The pink end reaction is beautifully clear and sharp. The second reading multiplied by the factor 0.0014 gives the amount of nitrogen in grams in 10 c.c. of the fluid.

It is then computed for the twenty-four-hour volume as for N, eliminated as ammonia. Example: It required 5 c.c. N/10 NaOH—5×0.0014=0.007. As originally 10 c.c. were diluted to 200, the 10 c.c. taken for titration would only be 1/20; hence 0.007×20=0.14 gram for 10 c.c. or 1.4 for 100 c.c. or 14 grams for 1000 c.c.

Urea Estimations

The amount of urea, which represents from 85 to 90% of the total nitrogen, is usually determined instead of the total N. The hypobromite and hypochlorite methods are, however, lacking in accuracy, and more exact methods of urea estimation are more time-consuming than the one just given for total N.

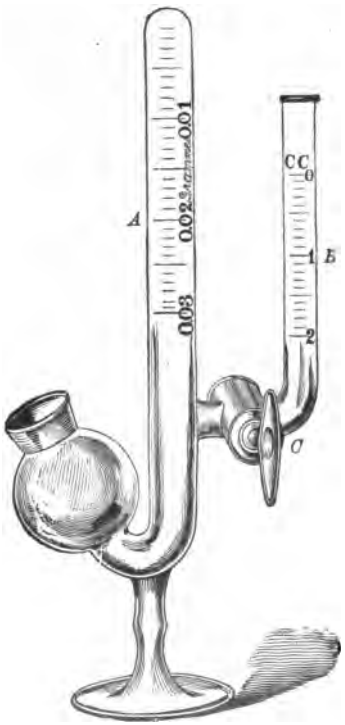


Fig. 142.—Doremus-Hinds Ureometer.

Probably the most convenient test for urea is the hypobromite method, using the Doremus ureometer with a side tube connected to the closed arm of the fermentation tube by a glass stop cock.

The reagent is prepared by taking 70 c.c. of a 30% stock solution of NaOH, diluting it with 180 c.c. water and then adding 5 c.c. of bromine, stirring until the bromine is dissolved. This solution if stored in a cool dark place will keep about one week.

The urine to be tested must be free from sugar and albumin and contain less than 1% of urea. Ordinarily the urine must be diluted two to four times to obtain a specimen containing less than 1%. In using this improved Doremus ureometer the closed portion of the U tube is filled with the hypobromite solution, and the urine introduced by allowing it to run in from the side tube by opening the glass cock arranged for that purpose. After the gas has risen and the instrument has stood for a short time the readings may be made in grams to the liter, or in percentage.

This urea determination is only a rough clinical one.

Urea Determination by Urease Method.—Put 1 or 2 c.c. of toluol into each of two 200 c.c. Erlenmeyer flasks; to one, add exactly 5 c.c. of a specimen and 100 c.c. of distilled water; to the other, one Urease-Dunning tablet, crushed and dissolved in about 5 c.c. of water, using a small glass mortar. Rinse the mortar with several portions of distilled water until about 100 c.c. have been introduced into second flask and then add exactly 5 c.c. of the urine specimen. Stopper both flasks with cork

and agitate contents. If time is not a consideration, allow flasks to stand at room temperature, at least eight hours, or use two tablets and digest at 40°C. for one hour. Rapid determination may be made by using but 1 c.c. of urine, two tablets and 100 c.c. of distilled water and digest between 40° and 50°C. for thirty minutes only.

After the elapse of proper time, titrate the two solutions to a distinct pink color with N/10 HCl and methyl orange. The amount of HCl required to neutralize the urease-treated specimen, less the amount required for the control, will give the urea content, estimated upon its equivalent in ammonium carbonate.

The controls of a large number of determinations made at one time may be titrated, as to existing alkalinity, with N/10 HCl and methyl orange, one after another, in the same flask, at the time the portions to be examined for urea are prepared in separate flasks. In such cases, a sufficient number of Urease-Dunning tablets, for all, may be rubbed up in a mortar with a measured quantity of distilled water and an aliquot portion, with 100 c.c. of distilled water, added to each of the separate specimens treated. See urease method for blood.

Gerhardt's Test for Diacetic Acid

Add a few drops of ferric chloride solution to 10 to 50 c.c. of urine as long as a precipitate continues to form. Then filter and to the filtrate add more ferric chloride solution. A bordeaux red color shows diacetic acid. The test is sensitive. As a control to show that the color is not due to drug elimination (antipyrine, salicylates, etc.) boil a specimen which gave the test for three to five minutes. If the color was due to drugs it will be obtained with a boiled sample while such treatment drives off the diacetic acid. In Hurtley's test add 2.5 c.c. HCl and 1 c.c. of 1% sol. of sod. nitrate to 10 c.c. urine. Shake and allow to stand two minutes. Now add 15 c.c. strong ammonia followed by 5 c.c. of 10% sol. ferrous sulphate. The slow production of a violet color shows positive test (two hours). Shows 1 part aceto-acetic acid in 50,000.

If the urine shows a well-marked Gerhardt reaction it is well to test for β-oxybutyric acid.

The following modification of Lange's test by Hart is a satisfactory one. The principle involved is the removal of acetone and diacetic acid by heat, then oxidizing β-oxybutyric acid to acetone with hydrogen peroxide and then testing for acetone.

Method: Take 20 c.c. of urine, dilute with an equal amount of water and add a few drops of acetic acid. Next boil in a beaker until the original amount of diluted urine is reduced to 10 c.c. (originally 40 c.c.). Dilute this evaporated urine with an equal amount of water, giving us 20 c.c. In each of two test-tubes put 10 c.c. of this 20 c.c. To one tube add 1 c.c. of hydrogen peroxide and warm gently, without boiling, for one minute; then cool. The other tube is left untreated. Next, to each test-tube add 10 drops of glacial acetic acid and 5 to 10 drops of a freshly prepared sodium nitroprusside solution and mix. Next carefully overlay each tube with about 2 c.c. of concentrated ammonia. If β-oxybutyric acid were present in the tube treated with the hydrogen peroxide and thereby oxidized to acetone a violet-red ring will develop at the point of contact while in the untreated tube there will be no such color ring.

A yellowish-brown ring from the presence of creatinin may show in the untreated

tube. It is well to allow the tubes to stand for three to four hours before finally reporting the absence of β-oxybutyric acid. It will probably show 0.2%.

Acetone.—To one-sixth of a test-tube of urine add a crystal of sodium nitroprusside. Make strongly alkaline with NaOH. Shake. The addition of a few drops of glacial acetic gives a purple color to the foam, if acetone is present.

Acetone is naturally present in exceedingly small quantity. It is very much increased in advanced cases of diabetes mellitus. It is also frequently found in scarlet fever, typhoid fever, pneumonia, nephritis, and severe anemias. It is often present after ether or chloroform anæsthesia.

Although the following test can be applied directly to the urine it is preferable to obtain distillates when possible and test these.

Gunning Test.—To about 5 c.c. of the urine add a few drops of Lugol's solution and then ammonium hydrate until a black precipitate forms. Allow to stand for some time, when, if acetone is present, a yellowish precipitate of iodoform crystals will separate. These should be identified by microscope. Iodoform crystallizes in some modification of the hexagonal plate, usually a six-pointed stellate form. If the crystals are not clearly defined, throw precipitate on filter, wash with a little water, and pour over it a little hot alcohol. To this filtrate add water, drop by drop, until a precipitate, which will consist of well-defined crystals of iodoform, is obtained. Examine this by microscope.

Never use an alcoholic solution of iodine in connection with this test.

Diazo Reaction.—To 5 c.c. sulphanilic acid solution (sulphanilic ac. 1 pt., HCl 50 pts., aq. 1000 pts.) add 2 drops of a 0.5% solution of sodium nitrate. Add an equal quantity (5 c.c.) of urine. Shake and add quickly 2 or 3 c.c. of ammonium hydrate. A carmine color, especially in the foam, shows a diazo reaction. If the reaction is positive, and the mixture is allowed to stand for twenty-four hours, a precipitate forms, the upper margin of which exhibits a green, greenish-black or violet zone.

Indican.—Take 10 c.c. urine and treat it with 1 c.c. of sol. of lead subacetate. Filter. Of this filtrate take 6 c.c. and treat with an equal amount of Obermayer's reagent; allow to stand for five minutes then shake gently with 2 c.c. of chloroform. Obermayer's reagent is strong HCl containing 2 parts of ferric chloride to the liter —0.1 gram to 50 c.c. of HCl.

A more exact method is to pour off the supernatant acid urine. Wash the chloroform with water, then pour off as much of the supernatant water as possible and add 10 c.c. of alcohol. A clear blue fluid results.

Urobilin.—Urobilin appears in considerable quantity in urine when there is much destruction of red cells, as in pernicious anæmia, internal hæmorrhage, and in malarial cachexia. The best test is that of Schlesinger. To the unfiltered urine add an equal amount of a saturated solution of zinc acetate in absolute alcohol. Shake, add a few drops of Lugol's solution and filter. Fluorescence in the filtrate shows the presence of urobilin. The degree of blood destruction is indicated by the intensity of the fluorescence.

Bile Pigments.—A satisfactory test is that of Rosin (Trousseau). Overlay 10 c.c. urine with about 5 c.c. of dilute tincture of iodine (1 to 10 of 95% alcohol). An emerald green ring at the point of contact shows the presence of bile coloring matter. For *Gmelin's test* pass the urine several times through the filter and then

touch the paper with a glass rod which has been dipped in a commercial HNO_3. A green color play shading to blue and violet shows bilirubin.

Bile Acids.—Bile pigments and bile acids usually occur together in urine. A very delicate and reliable test for bile acids is that of Oliver. Filter a specimen of urine until it is quite clear. Then make reaction acid provided the urine is not acid. The urine should be diluted with water until its specific gravity is below 1.008. The reagent is made as follows: Peptone 30 grains and salicylic acid 4 grains are dissolved in 8 ounces of distilled water containing ½ dram of acetic acid.

The solution is filtered until transparent.

For the test add 20 minims of the clear acid urine to 60 minims of the reagent. A milky turbidity indicates bile acids. If the turbidity disappears on shaking, the addition of more reagent will cause it to reappear.

Uric Acid in Urine

Normally we have from 0.2 to 2 grams eliminated in twenty-four hours depending on nature and quantity of protein intake. Nuclein rich foods, as sweetbreads, liver, etc., greatly increase the amount.

To determine the quantity add to 150 c.c. of the urine 50 c.c. of a reagent which consists of 500 grams ammonium sulphate, 5 grams of uranium acetate, and 60 c.c. of 10% acetic acid dissolved, with the aid of heat, in sufficient water (about 700 c.c.) to make 1 liter of solution. The mixture of urine and reagent is then filtered. To 134 c.c. of the filtrate (which represents 100 c.c. of the urine), contained in a beaker, add sufficient ammonium hydrate to make strongly alkaline, and let stand twenty-four hours. Filter, preferably through hardened filter, wash beaker, and precipitate thoroughly with 10% ammonium sulphate solution; open filter and with wash bottle wash precipitate back into beaker, using approximately 100 c.c. of water to do so, add 15 c.c. of concentrated sulphuric acid, and then immediately from burette add N/20 potassium permanganate solution until a pink color which persists for thirty seconds is obtained. The number of cubic centimeters of the potassium permanganate solution required is then multiplied by 0.00375; to this result add 0.003 for each 100 c.c. of filtrate and wash solution used. The final result is the quantity of uric acid in 100 c.c. of urine.

Chlorides

These are normally present in quantity corresponding to 10 to 15 grams of sodium chloride. Diet as well as certain pathologic processes, especially the latter, may cause marked deviation in quantity and as the deviation is usually very well marked, a method giving only fairly accurate results is sufficient for their estimation.

Dilute 5 c.c. of the urine, which should be free from albumin, with 50 to 75 c.c. of distilled water, add 10 to 15 drops of a solution of potassium chromate and then from a burette add N/10 $AgNO_3$ until a very slight pinkish tinge is obtained. The number of cubic centimeters of $AgNO_3$ required, multiplied by 0.00585 will give the quantity of sodium chloride to which the chlorine in 5 c.c. of urine is equivalent. Where a considerable degree of accuracy is demanded one should use either the Arnold or the Harvey modification of Volhardt's method.

Volhard-Arnold Method. Principle.—The urine is acidified with nitric acid and the chlorides precipitated with a measured excess of standard silver nitrate solution. The silver chloride formed is filtered off and in the filtrate the excess of silver nitrate is titrated back with standard ammonium thiocyanate solution. Ferric ammonium sulphate is used as an indicator. A red color due to the formation of ferric thiocyanate indicates that an excess of thiocyanate is present and the end point has been reached.

Procedure.—Place 5 c.c. of urine in a 100 c.c. volumetric flask, add 20–30 drops of nitric acid (sp. gr. 1.2) and 2 c.c. of a cold saturated solution of ferric ammonium sulphate (ferric alum). If necessary, at this point a few drops of 8% solution of potassium permanganate may be added to dissipate the red color. Now slowly add 20 c.c. of N/10 silver nitrate solution in order to precipitate the chlorine and insure the presence of an excess of silver nitrate. The mixture should be shaken continually during the addition of the silver nitrate solution. Allow the flask to stand ten minutes, then fill it to the 100 c.c. graduation with distilled water and thoroughly mix the contents. Now filter the mixture through a dry filter-paper, collect 50 c.c. of the filtrate and titrate it with N/10 ammonium thiocyanate solution. The first permanent tinge of red-brown indicates the end point.

Calculation.—The number of cubic centimeters of ammonium thiocyanate solution used indicates the excess of N/10 silver nitrate solution in the 50 c.c. of filtrate titrated. Multiply this reading by 2, inasmuch as only one-half of the filtrate was employed, and subtract this product from the number of cubic centimeters of silver nitrate (20 c.c.) originally used, in order to obtain the actual number of cubic centimeters of N/10 silver nitrate solution utilized in the precipitation of the chlorides in the 5 c.c. of urine employed.

To obtain the weight in grams of the sodium chloride in the 5 c.c. of urine used, multiply the number of cubic centimeters of the N/10 silver nitrate solution, actually used in the precipitation by 0.00585. From this figure the amount of sodium chloride excreted in twenty-four hours can easily be computed.

Formaldehyde in Urine after Administration of Urotropin

As formaldehyde fails to appear in the urine of possibly 52% of those taking urotropin as a genito-urinary antiseptic in quantities sufficient to inhibit bacteria growth (1–5000) the usual tests are too delicate. As a practical guide to efficient breaking up of urotropin the test proposed by Burnam is to be recommended.

To about 10 c.c. urine in a test-tube at body temperature add 3 drops of ½% solution of phenylhydrazin hydrochloride and 3 drops of a 5% solution of sodium nitroprusside. Finally allow a few drops of 20% solution of NaOH to run down the side of the test-tube and as this diffuses throughout the urine a deep purplish-blue color, rapidly changing to a dark green becoming lighter green and finally pale yellow will show if formaldehyde is being excreted in sufficient strength. In the absence of sufficient formaldehyde a reddish color develops which finally turns to a light yellow.

In order to obtain effect from urotropin it is necessary to have the urine acid. This is best accomplished, if an acid reaction is absent, by the administration of dihydrogen sodium phosphate (acid sodium phosphate). In carrying out Burnam

test albumin in the urine confuses the color reaction. By careful boiling to precipitate the albumin, then filtering, we avoid confusing colors.

Phenolsulphonephthalein Test for Renal Efficiency

Geraghty has recently stated that in 35 cases where an autopsy made it possible to verify the accuracy of this test the lesions revealed at autopsy corresponded closely with the results of the test. Again in 30 nephrectomies the conditions found were in accordance with the results of the test. The general opinion of those who have used the test is that it is more reliable than cryoscopy and far easier

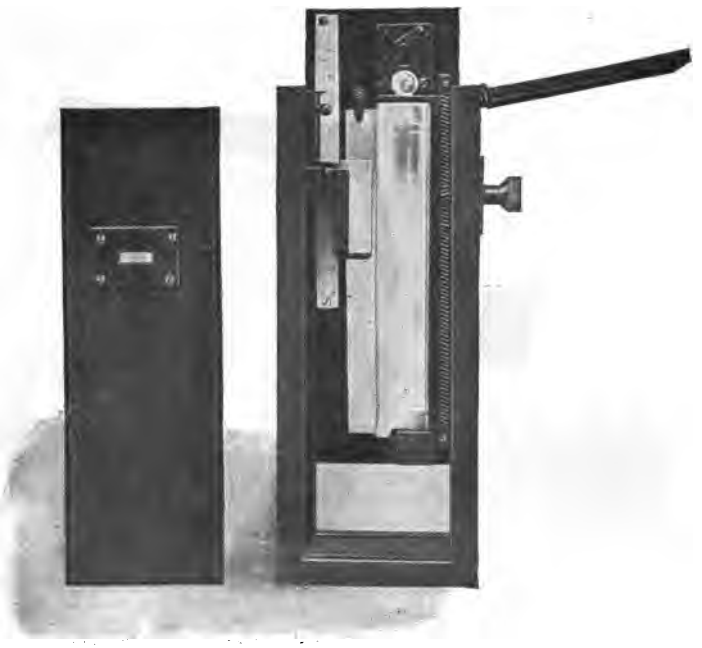


FIG. 143.—Rowntree and Geraghty modification of Hellige Colorimeter.

of application. The technic is as follows: One c.c. of the phthalein solution containing 6 mg. is injected intramuscularly or subcutaneously. The drug can be bought in ampules ready for use. About twenty minutes before injecting the drug the patient is given from 200 to 400 c.c. of water to drink. After the injection the bladder is emptied with a catheter and the time is accurately noted when the urine which subsequent to the emptying of the bladder and being allowed to drop into a test-tube containing 1 drop of a 25% sodium hydrate solution first shows a pinkish tinge. This is recorded as the time of appearance of the drug in the urine and normally is about ten minutes. The catheter is then withdrawn and the urine that

is passed in the first hour is collected by catheterization and subsequently that passed in the second hour. To each hour's specimen sufficient 25% sodium hydrate is added to give a purple-red color and the entire amount of the first hour's urine is then poured into a liter flask and made up to 1000 c.c. A similar treatment is employed for the urine of the second hour. The amount of drug eliminated in each hour is then determined by a colorimeter.

For the comparison standard 0.5 c.c. (3 mg.) of the phthalein solution is diluted to 1000 c.c. and made alkaline with 1 to 2 drops of 25% NAOH this gives a 50% solution of the drug dose (6 mg.).

In cases with marked impairment of renal function it may be advisable to dilute each hour's urine to 500 c c. instead of 1000 c.c., dividing the reading by 2.

The test may be fairly accurately carried out by having the patient void his urine instead of passing a catheter, separating that passed in the first hour from the urine of the second hour.

FIG. 144.—Dunning colorimeter.

It is also convenient to take one-tenth of the amount passed in the first and second hours and make up to 100 c.c. instead of the entire amount up to 1000 c.c.

Cabot has proposed the use of a series of 10 test-tubes containing solutions of the drug representing from 5% to 50% of the drug dose, each tube containing 5% more than the preceding one. These comparison solutions may be made up with the patient's urine obtained at the time of emptying the bladder so that the confusion which may obtain when water is used is avoided. It has recently been proposed to make the standards with water and use a piece of yellow glass for matching. The urine to be tested made up to 1000 c.c. as previously described is then poured into a test-tube of similar size and matched.

In normal cases Cabot got 46% of the drug eliminated in the first hour, the average for the second hour being 17%. The quantity of urine secreted in either

hour has no relation to the test, which is the percentage of drug eliminated. In cases with serious kidney disease the amount of drug eliminated in the first hour may range from 5 to 12%.

When the question of the kidney involved arises, the urine must be taken by ureteral catheterization or by a separator.

Dunning has devised a simple inexpensive colorimeter for the drug excretion determination in place of the more expensive colorimeters such as those of Duboscq and Hellige. A cut of the apparatus is given. As a rule, I make up my known standards with urine instead of water as giving better comparison and pour a darker known solution from a graduated cylinder into a Nessler jar as described under blood-sugar estimations.

F—CHEMICAL EXAMINATION OF GASTRIC CONTENTS

The test breakfast ordinarily used is that of Ewald (one shredded wheat biscuit or two small pieces of toast with 400 c.c. of water is what is usually given). This Ewald breakfast is a low-grade stimulant to acid production. It is given in the morning on an empty stomach. If at supper, the night before, the patient partake of raspberry jam the finding of the characteristic seeds in the stomach contents the next morning would be evidence of lack of motor activity. The Fischer meal which contains a 4-ounce Hamburg steak in addition to the water and toast of the Ewald is withdrawn after three hours.

The stomach tube is more easily passed if it be thoroughly chilled in ice water without the use of any lubricant.

The stomach tube should be passed one hour after the Ewald breakfast and if more than 50 c.c. of fluid be obtained it indicates stasis or hypersecretion.

Free HCl.—Filter the gastric contents and test first for free HCl. The most reliable and sensitive test is that of Gunsberg. The reagent, which should be freshly prepared, consists of phloroglucin 3 grams, vanillin 1 gram, and absolute alcohol 30 c.c. By mixing 2 drops of gastric juice and an equal quantity of Gunsberg reagent in a small porcelain dish and carefully heating above a flame we obtain a carmine red color if free HCl be present. A water-bath is preferable.

The Gluzinski test is of value in the diagnosis of gastric cancer. In this, three tests for free HCl are made, one from gastric contents and washings taken in the morning before taking any form of food. Then give the albumin of 2 hard boiled eggs, finely minced in 150 c.c. water. One hour later remove the contents and examine for free HCl. Then give a meal of broth, lightly broiled steak, mashed potatoes and bread and three hours later remove contents with stomach tube and test again for free HCl.

For lactic acid a modification of Strauss' method is quite satisfactory. Shake, in a test-tube, 5 c.c. of gastric contents with 20 c.c. of ether, allow to settle and pour off 5 c.c. of the supernatant ether into another test-tube. To this ether add 20 c.c. of water and 2 drops of a 1 to 9 solution of ferric chloride and shake well. The presence of 1% of lactic acid will give an intense greenish color.

Total Acidity.—Having determined the presence or absence of free hydrochloric or lactic acid, we should make a quantitative test of the various determinations of

the acidity of gastric juice (a modified Töpfer test). These are: 1. Free HCl. 2. Combined HCl. 3. Acid salts, and 4. Total acidity.

To 10 c.c. of filtered gastric contents, in a beaker, add 3 drops of dimethyl-amido-azo-benzol solution (a ½% solution in 95% alcohol). In the presence of free HCl the fluid becomes a rich carmine pink.

After reading the burette run in N/10 NaOH solution until the pink color is discharged and a light yellow color is obtained. This reading multiplied by 10 gives the amount of free HCl in degrees, a degree corresponding to 1 c.c. N/10 NaOH. Next add 6 drops of a ½% alcoholic solution of phenolphthalein to the light yellow fluid in the beaker. Again titrating the same preparation we add N/10 NaOH until a faint but distinct pink color is produced. The number of cubic centimeters added for the free HCl plus the number to give the pink color when multiplied by 10 gives the total acidity in degrees. (For example: 2.5 c.c. N/10 NaOH used to obtain yellow color—$2.5 \times 10 = 25$ or acidity due to free HCl. After adding the phenolphthalein, 4 c.c. N/10 NaOH required to produce pink color—$4 + 2.5 \times 10 = 65$ or total acidity in terms of acidity. This means that it would require 65 c.c. N/10 NaOH to neutralize 100 c.c. of gastric juice. A total acidity of 60 is about normal. To obtain percentage in HCl multiply by 0.00365; thus, $65 \times 0.00365 = 0.23\%$ HCl.)

Having determined the total acidity add 3 c.c. of 10% neutral calcium chloride solution to the gastric contents already in the beaker. As a result of the formation of acid calcium phosphate the pink color is discharged. Again add N/10 NaOH from the burette until the pink color is restored. The number of cubic centimeters used gives the amount of acid salts present.

From the figures for the total acidity subtract the sum of that for free HCl and for acid salts and the remainder will give the acidity due to combined HCl.

Wolff and Junghans' Test

This is an important test as distinguishing benign achylias from those associated with malignant disease of the stomach. Of course, where the carcinoma originates in the lesion of an ulcer or when small or in the region of the pylorus there may not be an achylia, free HCl being present.

While interpretation of increased albumin in those cases of carcinoma showing free HCl, owing to digestive action, would be difficult, this is less so in the typical case with its absent free HCl. To carry out the test it is best to give the patient only a light supper and follow this with mild cathartic the same evening. Give the Ewald test breakfast the next morning and withdraw contents in one hour. Filter the contents and of the filtrate deposit 1 c.c., 0.5 c.c., 0.25 c.c., 0.1 c.c., 0.05 c.c., and 0.025 c.c. in six test-tubes. Make up contents of each tube to 10 c.c. with distilled water. The dilutions will be from 1 to 10 in the first tube to 1 to 400 in the sixth tube. After mixing the tubes superimpose 1 c.c. of the reagent on each tube. The reagent is phosphotungstic acid 0.3 gram, concentrated HCl 1 c.c., 96% alcohol 20 c.c., distilled water to make 200 c.c.

Normal gastric juice gives a turbid albumin ring in the first two or occasionally in the third (1 to 40) tube. Turbidity in the 1 to 100 or over is suggestive of carcinoma.

The increase of albumin content in malignant achylias is thought to be due to the action of a specific ferment capable of acting on the protein content of the meal forming the soluble albumin we test for.

Pepsin

The determination of the quantity of pepsin is rather troublesome by the older methods, and in consequence a simple method, which also uses a protein, will be given. The protein used is edestin, a globulin obtained from hempseed. Dissolve 0.1 gram of the edestin in 100 c.c. of 0.1% HCl. (This is most quickly done by sprinkling the edestin over the surface of the acid.) In each of six small test-tubes place exactly 2 c.c. of the edestin solution (thus giving 2 mg. of the substance to each tube). Dilute 1 c.c. of stomach contents to 20 c.c. with water. To the above-mentioned test-tubes are added, respectively, 0.2, 0.4, 0.6, 0.8, and 1 c.c. of the diluted sample, the last test-tube being left as a blank. Shake each test-tube so that its contents will be mixed. Allow the tubes to stand for thirty minutes. Then to each tube add 0.3 c.c. of a saturated solution of NaCl. In those tubes which do not contain sufficient pepsin to digest 2 mg. of edestin a white cloud or precipitate will be produced while all others remain clear. (The blank or sixth tube must give a copious precipitate or the edestin solution is worthless.) The tube containing the smallest quantity of the dilute sample and giving no cloud or precipitate is selected. Then by the formula 2 x dilution divided by the volume of dilute sample present is obtained the number of milligrams of edestin that will be digested by 1 c.c. of the undiluted sample. This quantity normally is said to be 100. If all but the sixth tube failed to give the cloud or precipitate a greater dilution of the sample must be made and the test repeated. The edestin solution rapidly decomposes and must be made fresh when needed.

Mett's Method for Quantitative Estimation of Pepsin.—Capillary glass tubes, 1 or 2 mm. in diameter are filled with white of egg, and after plugging one end with bread crumbs the tubes are placed in water at 97° or 98°C. for five minutes. The tubes are sealed with plasticine or sealing wax. For the test dilute 1 c.c. of gastric juice with 15 c.c. N/20 HCl and put some into a test-tube. Then file off two 2-cm. columns of the capillary tube with coagulated egg albumin. Place test-tube with its albumin tubes in incubator for twenty-four hours. Then measure the digested ends of the two capillary tubes and take average. The square of this figure gives the number of units of pepsin in the 1 to 16 dilution of gastric juice and when multiplied by 16 the number of units in 1 c.c. of gastric juice. Normal variation is 8 to 100 units.

G—CHEMICAL EXAMINATION OF DUODENAL JUICE

The general discussion of the subject of the examination of the duodenal juice is taken up in Part IV. As stated there the lipase tests are rather unreliable so only tests for amylase (amylopsin) and protease (trypsin), as recommended by Myers and Fine are given.

Wohlgemuth Method for Amylase.—Place 5 c.c. of 1% soluble starch solution in each of six small test-tubes. Tube I serves as a control, and to each of the other five

tubes add 0.05, 0.1, 0.25, 0.5, and 1.0 c.c. of the duodenal juice diluted one-half with distilled water.

Place in 37°C. incubator for one-half hour, add cold water to almost fill the tubes and then several drops of N/10 iodine. The positive tube will show an entire absence of blue color. The amylase strength is represented by the number of cubic centimeters of starch solution 1 c.c. of undiluted juice can digest. If 1 c.c. of undiluted duodenal juice digests 5 c.c. of starch solution the amylase strength is 5; if 0.1, 50. The average normal is about 50.

Gross Casein Method for Trypsin.—Put in each of six test-tubes 5 c.c. of 0.1 % solution of casein in 0.1 % sodium carbonate solution and add to each one, except tube I, which serves as control, the same amount of diluted duodenal juice as given above for amylase. Incubate for fifteen minutes at 38°C. and acidify with a few drops of dilute acetic acid. The activity is calculated as for amylase and is normally from 4 to 10. *White* makes a 1% dilution of the duodenal fluid with water and puts 10 c.c. of 0.1% casein solution in each tube. The tube with 0.1 c.c. of diluted fluid would therefore be a 1 to 10,000 dilution and that with 1 c.c. a 1 to 1,000. Normally the 1 to 10,000 tube remains cloudy. Clouding of the 1 to 1,000 tube would show marked lowering of trypsin.

H—DISINFECTANTS AND INSECTICIDES

By disinfection is meant the destruction of injurious bacteria.

Sterilization is where all living things are destroyed.

Germicides are substances which kill bacteria while antiseptics are those which are inimical to the growth of bacteria.

Formalin is antiseptic in 1–50,000 dilution but germicidal only in 1–20.

Deodorants may or may not be antiseptic or germicidal. An insecticide may or may not be a germicide and *vice versa*.

In disinfection we must consider

(1) Strength of solution. It must always be kept in mind that the strength of a germicide solution when added to an equal amount of material to be disinfected is reduced in strength one-half. Thus 1 pint of a 5% comp. cresol solution added to 1 pint of fæcal material has only a 2½% disinfecting effect.

(2) Time of application. A common mistake is to consider a few minutes as sufficient for contact of germ-containing material with the disinfectant. In the fæces-cresol mixture above noted the action of the disinfectant should continue at least one hour before emptying the vessel.

(3) Nature of medium in which disinfectant acts. Bacteria are much more resistant to germicide agents when contained in solution rich in organic matter than when suspended in pure water. Thus Liq. Cresol. Comp. has a phenol coefficient of 3 without organic matter and only 1.87 with organic matter.

(4) Temperatures. Disinfecting solutions show greater power as the temperature rises, and act less efficiently in the cold. A body temperature (39°C.) is a good one.

By Coefficient of Inhibition we mean time and concentration necessary to prevent development of bacteria.

By Inferior Lethal Coefficient we mean time and concentration necessary to kill nonspore-bearing bacteria.

By Superior Lethal Coefficient we mean time and concentration necessary to kill spore-bearing bacteria.

U. S. Hygienic Laboratory Phenol Coefficient.—*In determining the strength of a disinfectant* it is compared with that of phenol (commercial carbolic acids vary in their phenol content). If more powerful than phenol the coefficient will be greater than 1. In the U. S., disinfectants are rated according to the "Hygienic Laboratory Phenol Coefficient."

The determinations *when organic matter was not present*, were conducted as follows:

"The experiments were done at a temperature of 20°C. maintained by means of a water-bath. The quantity of each dilution of disinfectant and phenol used in each experiment was 5 c.c. The culture used was *B. typhosus*, twenty-four-hour extract broth, filtered. The seeding tubes containing the disinfectant dilutions and the filtered broth culture of *B. typhosus* were placed in the water-bath and allowed to reach the temperature of 20°C. before starting the experiment.

"The seeding tubes were inoculated successively every fifteen seconds with 0.1 c.c. of the typhoid culture. Each tube was gently shaken after it was inoculated. At the end of each two and one-half minute period, for fifteen minutes, plants were made from the seeding tubes into tubes of extract broth. The medium used was standard extract broth having a reaction of +1.5. The quantity of broth in each tube was approximately 10 c.c.

"The tubes were incubated at 37°C. for forty-eight hours, at the end of which the results were recorded.

"*To determine the coefficient*, the figure representing the degree of dilution of the weakest strength of the disinfectant that kills within two and one-half minutes is divided by the figure representing the degree of dilution of the weakest strength of the phenol control that kills within the same time. The same is done for the weakest strength that kills within fifteen minutes. The mean of the two is the coefficient.

"When the determinations were made as to efficiency *in the presence of organic matter* a stock solution of 10% peptone and 5% gelatin in water was made up and of this 1 c.c. was put in a tube and then inoculated with 0.1 c.c. of typhoid culture. Then 4 c.c. of the varying phenol and disinfectant dilutions were added and the tests conducted as above."

Disinfectants may be (A) Physical, (B) Gaseous, (C) Chemical

(A) **Of the physical disinfectants** we have

(1) Sunlight. The red and yellow rays practically inert. The violet and ultra-violet most active. Direct sunlight kills plague bacilli in less than one hour—typhoid bacilli in six.

(2) Burning. Very efficient but expensive.

(3) Boiling. Especially in carbonate of soda solution for about one hour is a very efficient disinfectant. Nonspore-bearing bacteria are killed almost instantly by a boiling temperature. One must remember that the boiling temperature is lower at mountainous elevations.

(4) Steam. Extremely efficient. The condensation of the steam on the object to be sterilized gives off latent heat and produces a vacuum.

(B) **Of the gaseous disinfectants** we have the very efficient germicide formaldehyde gas and the weakly germicidal, but potent insecticide, sulphur dioxide.

Formaldehyde gas is practically valueless as an insecticide.

Bromine, chlorine and hydrocyanic acid gas have a certain degree of efficiency but are not of practical application. Hydrocyanic acid gas is especially dangerous on account of its extreme toxicity.

(1) **Formalin.**—This is a 40% solution of formaldehyde gas, but is as a rule of less strength from evaporation or otherwise. Formaldehyde is efficient as a surface disinfectant when the temperature is above 50°F. and the air contains at least 60% of moisture. It is not efficient in cold dry rooms. Owing to its lack of penetrating power it is not efficient for the disinfection of mattresses, or similar articles. To prepare a room for disinfection we must measure the cubic space to ascertain the necessary amount of formalin to use and stuff up or better paste up with newspaper all cracks and openings.

In the production of formaldehyde gas the more expensive autoclaves and lamps have largely been replaced by the simple formalin permanganate method. In this one pours 500 c.c. of formalin on 250 grams of potassium permanganate for each 1000 cubic feet with six to twelve hours' exposure.

In employing this method, take a pan partly filled with water. Place in this a second metal or glass receptacle containing the permanganate. Then pour the formalin on the permanganate crystals. The gas is generated in great amount in a few seconds. The receptacle containing the permanganate and formalin should be large enough to contain 10 times the volume of formalin, as there is a tendency for the mixture to foam over the sides of the dish.

Another practical method is the **formalin-sheet-spraying** one. The formalin (40%) should be sprayed on sheets suspended in the room in such a manner that the solution remains in small drops on the sheet. Spray not less than 10 ounces of formalin (40% formaldehyde) for each 1000 cubic feet. Used in this way a sheet will hold about 5 ounces without dripping or the drops running together. The room must be very tightly sealed in disinfecting with this process and kept closed not less than twelve hours. The method is limited to rooms or apartments not exceeding 2000 cubic feet. The formalin may also be sprayed upon the walls, floors, and objects in the room.

Paraform Lamps.—For single rooms the use of the paraform lamp is quite convenient. Special lamps can be obtained to burn the paraform tablets or a pint tincup will suffice for the heating of 1 ounce of paraform. The lamp or alcohol flame under the receptacle must not be high enough to ignite the paraform which burns readily and in so doing does not give off formaldehyde gas. One ounce of paraform is sufficient for a space of 500 cubic feet. One can dissolve 2 ounces of paraform in 8 ounces of boiling water and then pour this over 4 ounces of potassium permanganate in a 2-gallon pail.

N. Y. Health Department Method.—After a prolonged series of tests the N. Y. Department of Health gave preference to the following formula:

Paraformaldehyde 30 grams, potassium permanganate 75 grams, water 90 grams. The chemicals are mixed in a deep quart pan and the water is added and the mixture stirred. The evolution of gas is slow in starting but is complete in five to ten minutes.

It was found that 87% of the gas was evolved and the quantities given above suffice to disinfect 1000 cubic feet in four hours. It is well to put the small pan containing the chemicals in a larger one to prevent danger of fire and soiling of the floor by the frothing of the mixture.

Sulphur Dioxide.—Sulphur dioxide is fairly efficient, but requires the presence of moisture. It is only a surface disinfectant and is lacking in penetrating properties. An atmosphere containing 4.5% can be obtained by burning 5 pounds of sulphur per 1000 cubic feet of space. This amount requires the evaporation or volatilization of about 1 pint of water. Under these conditions the time of exposure should be not less than twenty-four hours for bacterial infections. A shorter time will suffice for fumigation necessary to kill mosquitoes and other vermin. Dry sulphur dioxide produced by burning 2 pounds of sulphur for each 1000 cubic feet of space will answer for this purpose. An exposure of from two to three hours is sufficient.

The sulphur may be burned in shallow iron pots (Dutch ovens), containing not more than 30 pounds of sulphur for each pot, and the pots should stand in vessels of water. The sulphur pots should be elevated from the bottom of the compartment to be disinfected in order to obtain the maximum possible percentage of combustion of sulphur. The sulphur should be in a state of fine division, and ignition is best accomplished with alcohol (special care being taken with this method to prevent damage to cargo or vessel by fire), or the sulphur may be burned in a special furnace, the sulphur dioxide being distributed by a power fan. This method is peculiarly applicable to cargo vessels.

Liquefied sulphur dioxide may be used for disinfection in place of sulphur dioxide generated as above, it being borne in mind that this process will require 2 pounds of the liquefied gas for each pound of sulphur, as indicated in the above paragraphs.

Sulphur dioxide is especially applicable to the holds of vessels or to apartments that may be tightly closed and that do not contain objects that would be injured by the gas. Sulphur dioxide bleaches fabrics or materials dyed with vegetable or aniline dyes. It destroys linen or cotton goods by rotting the fiber through the agency of the acids formed. It injures most metals. It is promptly destructive of all forms of animal life. This property renders it a valuable agent for the extermination of rats, insects, and other vermin. Sulphur dioxide is a germicide only in the presence of moisture, and even then will not kill spore-bearing organisms. If clothing is washed immediately after sulphur disinfection the rotting effect will be greatly lessened. If used in spaces containing machinery all metal parts should be coated with vaseline.

Chemical Solutions

Bichloride of mercury is usually sold in the form of antiseptic tablets. As a disinfectant for the infectious diseases it is usually used in a strength of 1–1000. The solution should be made in a wooden or earthenware vessel. As bichloride forms inert albuminates it should not be used in the disinfection of sputum, fæces or any albuminous excreta. It must be remembered that bichloride is a mordant so that any stains in soiled clothing will remain permanent. For disinfection of clothing the material should be left in 1–1000 bichloride for one hour. Dishes for food should never be disinfected in bichloride on account of the danger from poisoning.

Floors and walls may be disinfected with 1–1000 bichloride applied with a mop. Allow the solution to dry on the floor or walls.

Formalin.—A 5% solution of commercial formalin in water (50 c.c. formalin 950 c.c. water) makes a satisfactory disinfectant for soiled clothing. It is also valuable for albuminous material. The disinfectant must act in a strength of 5% so that if 1 pint of fæces is to be disinfected we should add 1 pint of a 10% formalin solution and allow it to act for one hour.

Carbolic Acid.—It is soluble in water to the extent of about 5% and in such strength it is an efficient disinfectant. The solution should be made with hot water.

In standardizing disinfectants carbolic acid is used as the standard. It however is expensive and there is often difficulty in making up satisfactory solutions. More efficient and more convenient is the **Liquor cresolis comp. U. S. P.** This may be prepared by mixing up equal parts of cresol and soft soap as noted on page 15. This has a value according to tests made in the Hygienic Laboratory of 3, making it in tests without organic matter three times as efficient as carbolic acid. Under similar conditions lysol had a value of 2.12, creolin 3.25 and trikresol of 2.62.

Equal parts of a 5% solution of Liq. Cresol. Comp. and the fæces, urine or sputum to be disinfected is satisfactory for disinfection provided the mixture is allowed to stand for one hour. Here we would have the effect of a 2½% solution. Liq. Cresol. Comp. (5%) is an excellent disinfectant for contaminated bedclothing, etc. It is also most suitable for the disinfection of floors and walls.

Sulphate of Copper.—This salt has a remarkable effect on certain species of algæ so that in strengths of 1 to 1,000,000 it is destructive. In 1 to 400,000 it will kill typhoid bacilli in twenty-four hours in water that is not too full of organic matter.

Hydrogen Dioxide.—A 2% solution will kill anthrax spores in three hours. It is useful in treatment of anaerobic infections, as gas bacillus ones. When hydrogen dioxide is used in the presence of blood or pus, the catalase of the latter rapidly decomposes the H_2O_2 so that the disinfecting power rapidly disappears.

Chinosol.—This is a derivative of quinoline, a coal-tar product. It is a yellow powder readily soluble. It does not coagulate albumin and leaves no stain on clothing. It is efficient in a strength of 1 to 500 or 1 to 1000. It would seem that it is efficient as an antiseptic rather than as a germicide in which latter effect it is inferior to phenol.

Lime.—It must be remembered that air-slaked lime is inert as a disinfectant. For disinfecting fæces freshly prepared milk of lime is excellent. It is made by mixing unslaked lime with four times its volume of water. An equal quantity should be added to the fæces to be disinfected.

Chlorinated Lime.—This can be purchased in air-tight containers and when the package is opened it should give off a powerful odor of chlorine.

For a working disinfectant solution add 1 pound to 4 gallons of water. This is satisfactory for mopping floors and for disinfecting fæces, sputum and urine, equal parts of the excreta and disinfecting solution being mixed and allowed to stand for one hour.

Chlorine Gas.—In the treatment of waters contaminated with the organisms of the typhoid-colon group we depend almost entirely on the action of free chlorine usually as supplied from eylinders of liquefied gas. We also employ the gas as liberated from chlorinated lime or sodium hypochlorite. The destruction

of typhoid, paratyphoid or dysentery organisms as well as members of the Gärtner group is brought about by the action of from one-half to one part of free chlorine per million parts of water. Where water is turbid preliminary treatment with some precipitating agent as alum is required. Lelean recommends the addition of 2 grams of good quality bleaching powder (chlorinated lime) to the contents of an ordinary water cart which holds 110 gallons of water. This is actually about 1⅛ parts per million of chlorine so that allowing for possible deterioration we can count on 1 part per million being operative. Other agents used for disinfection of small quantities of water are "halazone," sodium bisulphate and calcium permanganate. Halazone tablets have been given excellent results in the sterilization of the contents of drinking bottles. It must always be remembered that boiling the water is the method of sterilization to be employed when practicable.

Eusol.—A solution containing 0.27% hypochlorous acid and known as eusol has been highly recommended in the treatment of gas gangrene wounds. To make it put 12.5 grams chlorinated lime (bleaching powder) in a Winchester quart flask and cover with a liter of water. After thorough shaking add 12.5 grams of boric acid. After shaking the mixture should stand for a few hours and then be filtered through cotton wool. The clear solution is *eusol*. It must be kept in tightly closed bottles.

Chloramine-T.—This chlorine antiseptic is more stable than hypochlorite solutions and can be used in greater concentration. It is nontoxic and readily soluble in water. It is usually used in 2% solution in the treatment of wounds. Gauze which has been impregnated with a 5% solution and dried can be used in light packing of wounds. In the eye, 0.1% (1–1000) in normal saline is efficacious and nonirritating.

Dichloramine-T.—This, like chloramine-T is a crystalline substance, but is practically insoluble in water. It is a very active germicide. In use it is dissolved in chlorinated eucalyptol or better still chlorinated paraffin wax. For treatment of infected wounds it is used in 6.5 to 10% strength, the chlorinated oil solution of the antiseptic being sprayed on the wound or gauze covering the wound.

Acriflavine.—Of the dyestuffs recommended as germicides this is better adapted to the purpose than malachite green or brilliant green. Acriflavine, or flavine as it is also called, acts more efficiently in serum mixtures than in aqueous ones and is less injurious to tissue than most other antiseptics. It is generally used in 1–1000 solution in salt solution and makes a good wet dressing when gauze is soaked in such a solution.

Dakin's Solution.—The best known and most widely used of the disinfectants of the chlorine group is a neutral sodium hypochlorite solution called Dakin's solution. This contains about 0.5% NaClO in a neutral solution. While *eau de Javel* is also a solution of sodium hypochlorite it is irritating on account of its containing an excess of alkali, the caustic action of which has an unfavorable action on wounds. In eusol the boric acid tends to neutralize this prejudicial alkalinity sufficiently to enable the buffer salts of the body fluids to maintain a proper neutral state.

The method of preparing Dakin's solution in the chemical laboratory of the Naval Medical School is as follows:

As a sample of chlorinated lime containing not less than the equivalent of 25% of sodium hypochlorite is the prime requisite and as samples of this substance vary

greatly in this respect, an assay is the first step in the process. This is carried out as follows: Obtain a representative sample by taking a portion from different parts of the container, then mix thoroughly in a mortar. Quickly weigh out 10 grams, place in a bottle and pour upon it 500 c.c. of water, stopper the bottle and allow to stand, with occasional shaking, for one hour. Filter a portion of this solution through a dry filter and into a dry flask until from 75 to 100 c.c. of filtrate are obtained—call this filtrate A. Stopper the flask and keep it so. Now into a 100 c.c. volumetric flask measure exactly 10 c.c. of the filtrate add 20 c.c. of a 10% solution of potassium iodide, then 2 c.c. of glacial acetic acid, mix, and allow to stand for a few minutes, then add water to make 100 c.c. of solution and then thoroughly mix—call this solution B. Fill a burette with this solution. In a beaker of about 75 c.c. capacity place exactly 5 c.c. of N/10 sodium thiosulphate, add about 2 c.c. of starch paste and from the burette add solution B until a faint blue color is produced. Each c.c. of N/10 thiosulphate = 0.003723 gram sodium hypochlorite and 5 c.c. will = 0.018615 gram. Let it be supposed that it was necessary to add 28 c.c. of solution B from the burette to bring about the end reaction. Then the 28 c.c. contained the equivalent of 0.018615 gram of sodium hypochlorite. The 100 c.c. of solution B contains the equivalent of 0.0665 gram sodium hypochlorite. This 100 c.c. of B solution was made from 10 c.c. of solution A. Ten c.c. of solution A contains 0.2 gram of the sample. One hundred grams of the sample will therefore contain the equivalent of 33.25 grams sodium hypochlorite or 33.25%.

Having obtained a satisfactory sample the solution proper is prepared as follows: In a jar, bottle or other vessel of 12 to 15 liters capacity place 200 grams of the chlorinated lime and pour on it 5 liters of water. Allow to stand in a cool place for two or three hours with occasional stirring. In another 5 liters of water dissolve 80 grams of dry sodium carbonate and 100 grams of sodium bicarbonate. Add this solution to that of the chlorinated lime. A gelatinous precipitate which does not readily settle, will form. This must be converted into a granular condition by vigorous stirring or shaking. The container holding it must be closed in some manner, placed in a cool place and allowed to settle. The clear supernatant fluid is then syphoned off into a large bottle. (It is well to measure the solution as it is drawn off for its volume must be known.) Call this solution C.

The strength of the solution must now be determined. To do this proceed as follows: Place exactly 10 c.c. of this solution in a 100 c.c. volumetric flask, add 20 c.c. of a 10% solution of potassium iodide, then 2 c.c. glacial acetic acid and allow to stand for a few minutes. Now add water enough to make 100 c.c. and mix—call this solution D. Place in a burette and titrate against 5 c.c. of sodium thiosulphate just as was done with solution B in the assay of the sample. Let us suppose that it required 29 c.c. to bring about the end reaction. Five c.c. of the thiosulphate = 0.018615 sodium hypochlorite. Then 29 c.c. of D contains this quantity of the hypochlorite; 100 c.c. will therefore contain 0.0642 gram. But this 100 c.c. of solution was made from 10 c.c. of solution of C. Ten c.c. of solution C then contains 0.0642 gram of the hypochlorite; hence 100 c.c. contain 0.642 gram or 0.642%.

This solution is too strong as not more than 0.5% is desired. The amount of dilution necessary is determined by the proportion 100 : 0.642 : : X : 0.5 whence X = 77.88; that is each 77.88 c.c. must be diluted to 100 c.c. Then to 7788 c.c. add 2212 c.c. of water to make 10,000 c.c. or 10 liters of solution. Having made the

necessary dilution its strength is determined as above in order to avoid mistakes. If found correct a little of the sample is placed in a beaker and a little phenolphthalein powder sprinkled upon it. The solution will not turn red if it has been properly prepared. Now, as a preservative, add 1 c.c. of a 0.5% solution of potassium permanganate to each liter of the finished product. It is now placed in well stoppered bottles and stored in a cool, dark place. It is better to store in a number of small bottles than in one large container as air space above the solution leads to rapid deterioration.

It must be borne in mind that in this work solutions with which we deal are readily decomposed and should be kept in stoppered containers at all times.

The starch paste used as indicator is made by mixing one gram of starch with about 10 c.c. of water and pouring it into 200 c.c. of boiling water.

The N/10 sodium thiosulphate is made by dissolving 24.8 grams of the substance in sufficient water to make 1000 c.c. of solution. This substance is also called sodium hyposulphite and has the formula $Na_2S_2O_3.5H_2O$.

Insecticides

The following notes are taken chiefly from the U. S. P. H. Service directions.

Sulphur Dioxide—obtained as described above—destroys all animal life.

In the case of vessels, when treated for yellow fever infection, the process shall be a simultaneous fumigation with sulphur dioxide, 2% volume gas, and two hours' exposure in order to insure the destruction of mosquitoes.

In the case of vessels when treated for plague the process with sulphur dioxide shall be as follows:

Without cargo: The simultaneous fumigation with sulphur dioxide gas not less than 2% for six hours' exposure.

With cargo: Fumigation with sulphur dioxide gas, 4%, six to twelve hours' exposure, according to stowing.

Infected vessels may require partial or complete discharge of cargo, and fractional fumigation for efficient deratization.

Pyrethrum.—The fumes of burning pyrethrum may be used to destroy mosquitoes in places where there are articles liable to be injured by the use of sulphur.

Four pounds per 1000 cubic feet space for two hours' exposure will kill all, or practically all, of the mosquitoes but precautions should be taken to sweep up and destroy any that may have escaped.

Pyrethrum stains walls, paper, etc.

The Oxides of Carbon, as used at Hamburg, are efficient to destroy rats but do not kill fleas or other insects. They are obtained by burning carbon, coke, or charcoal, in special apparatus, and the gas as produced consists of about 5% carbon monoxide, 18% carbon dioxide, and 77% nitrogen.

Twenty kilos of carbon, coke, or charcoal are used for every 1000 meters of space. The gas is allowed to remain in the ship for two hours and from seven to eight hours are allowed for it to leave it. This is about equivalent to 1⅓ pounds of carbon (coke) to 1000 cubic feet of air space. As this gas is very fatal to man and gives no warning of its presence, being odorless, a small amount of sulphur dioxide should be added to give warning of its presence. As it does not kill fleas it cannot

be depended on for complete work, where there is evidence of plague among rats on the vessel, as the infected fleas would infect the rats coming aboard after the deratization.

The articles named as disinfectants which can obviously destroy animal life can be used for that purpose when applicable, as steam for bedding, fabrics, etc. Formaldehyde is not applicable for this purpose.

Pulicides.—For fleas the best insecticides are (1) crude petroleum (fuel oil) which is at times called Pesterine, (2) an emulsion of kerosene oil made as follows: kerosene 20 parts, soft soap 1 part and water 5 parts. The soap is dissolved in the water by aid of heat and the kerosene oil gradually stirred into the hot mixture.

For *cockroaches* there is nothing so good as sodium fluoride. By sprinkling the powder about the haunts of the cockroaches they are gotten rid of in a few days.

Pediculicides.—Owing to their great importance in transmitting typhus fever, trench fever and relapsing fever the destruction of lice is a vital consideration.

While the body louse is the important transmitting agent, the head louse and possibly the crab louse should also be destroyed.

The subject of pediculosis has been much discussed on account of its importance among the troops in the European war. In Shipley's book on the "Minor Horrors of War" the following methods of destroying lice are given. *Pernet* gives the following instructions for the body louse.

1. All body and bed-linen and clothes should be baked or sterilized by boiling.

2. Unguentum staphisagriæ should be applied to neck-bands of vests and shirt in the region of the neck.

3. Alkaline baths to soothe the irritated skin.

Flowers of sulphur sprinkled in the bed and in the clothes is very useful.

Major Lelean recommends: A 2-inch loose-woven bandage is made into a tubular bag. Into this bag 2 teaspoonfuls of the following powder is poured and evenly distributed:

Naphthalene	96%
Iodoform	2%
Creasote	2%

The bag is then tied round the waist, and it is said that all lice are killed within twenty-four hours.

Moorhead advises the dusting of flowers of sulphur on the clothes but many reports indicate the inefficiency of this method.

For head lice Pernet recommends:

1. Prevention: hair to be kept close cropped and clean.

2. For the nits: wipe them off with a solution of 1 in 30 carbolic acid.

3. For the lice themselves: Unguentum hydrargyri ammoniat. dil. (gr. x to 1 oz.), or any fatty, sticky body well rubbed into the back of the head. Paraffin lamp-oil (kerosene) also good, but not to be used near a naked flame or light.

Blanchard considers camphorated alcohol or warm vinegar containing 1 to 1000 of corrosive sublimate as useful for head lice. He also suggests the fumigation of clothes with tobacco as valuable for body lice.

Castellani and Jackson have gone most extensively into the matter of louse destruction. Their conclusions are as follows: 1. In regard to solid and liquid insecti-

cides, the substances which have been found to be deleterious to body-lice are, in the order of their efficiency: Kerosene oil, vaseline, guaiacol, anise preparations, iodoform, lysol, cyllin and similar preparations, carbolic acid solution, naphthaline, camphor.

Pyrethrum has a very feeble action on lice, while boric acid, sulphur, corrosive sublimate, and zinc sulphate, when used in powder form, have apparently no action whatever. As regards bedbugs, kerosene oil is the best insecticide. Next to it comes guaiacol, one of the most active drugs of those tried.

2. For use against lice on a large scale, as among troops and prisoners, perhaps the best insecticide powder is naphthaline. This substance has a lower licecide action than kerosene oil, guaiacol, iodoform and anise preparations, but it has less displeasing odor than the first three named. In stored blankets and clothing it is also practicable and of use, as frequently lice are found upon the clothing and blankets stored through the summer.

Medical Director H. G. Beyer, U. S. N., gives the following description of the method of louse destruction employed by *Lenz* at the prison camp at Puchheim. On the arrival of a large transport of prisoners, the clothes they wear on their persons are, first of all, subjected to steam-disinfection, whereby a great mass of lice, including eggs, are destroyed. The hair on the bodies of the men is then shorn with a machine, and they are lathered over with soft soap and put into a bath. The now disinfected clothes are put on again and treatment with naphthalin is begun under the supervision of the sanitary police. Every person, at bedtime, has a handful of finely powdered naphthalin put into his clothes, introduced through the opening at the neck. He is made to sleep that night with all his clothes on him. The body heat causes the naphthalin to evaporate, the vapors killing not only the remaining lice, but also most of the remaining eggs. If this treatment is repeated twice more, regardless of whether living lice are found or not at the time, a thorough and complete louse disinfection will be assured. Lenz does not mention any disagreeable effects of the naphthalin vapors on the men themselves. When dealing with transports of smaller numbers of individuals, Lenz states that he has succeeded even without the employment of steam-sterilization of the clothes. The advantages of the naphthalin method as used by him are given as follows:

1. That it is cheaper than any other method, its cost per person being 1¼ cents.
2. That it does not interfere with the service efficiency of the men.
3. That it requires neither special apparatus nor places.
4. That it does not injure clothing.
5. That it is absolutely noninjurious to the health of the men.

N.C.I. Mixture.—It has been found that lice on clothing removed from the body may remain alive nine days and their eggs as long as forty days. It is, therefore, very important to subject the clothing of vermin-infested men to steam or to other disinfection. Boiling clothing for four or five minutes kills the eggs as well as the lice, as will also soaking in a 5% compound cresol solution for an hour. There are many methods of ridding the individual from lice, the most vaunted being the N.C.I. powder, which contains 96% commercial naphthalene, 2% creosote and 2% iodoform. This should be dusted into the shirt and drawers every four or five days. Magnesium silicate can be substituted for the iodoform. The N.C.I. mixture is rather too irritant for the region of the crotch. The mixture should be kept in

sealed tins. It has also been recommended to wring out the underclothes in 5% compound cresol solution, then drying thoroughly.

Moore has recently written an article on louse control in which he states that a powder of talc 20 grams, creosote 1 c.c. and sulphur 0.5 grams is six times as efficient as N.C.I. causing less irritation to the skin and being dry is easier to apply. He also calls attention to the value of chloropicrin as a fumigating agent. It is quite volatile and kills lice with fifteen minutes' exposure and eggs in all stages of development in thirty minutes. The clothing can be treated in a galvanized iron garbage can sprinkling 4 c.c. of chloropicrin to each cubic foot of space. Chloropicrin has great penetrating power. The effect of the fumigant is increased by heating the can container.

Raticides.—For exterminating rats and in this way secondarily the rat-fleas, besides the ordinary poisons such as As, P, etc. Rucker has recommended a poison composed of plaster of Paris, 6 parts, pulverized sugar 1 part and flour 2 parts. This mixture should be exposed in a dry place in open dishes. To attract the rats the edge of the dish may be smeared with the oil in which sardines have been packed.

Larvicides.—Wise and Minett report good results from the use of crude carbolic acid as a larvicide for mosquitoes. They added about 1 teaspoonful for each 2 cubic feet of water in the pool. Of course, the ordinary method for destroying mosquito larvæ is by covering the surface of the water in the cistern or pool with a layer of petroleum.

I—ANATOMICAL AND PHYSIOLOGICAL NORMALS

In examinations in the pathological or chemical laboratory the following may be considered approximately as normal findings:

A. Anatomical normals. Averages.

Adrenals. Length, 2.4–2.8 inches (6–7 cm.). Breadth, 1.2–1.4 inches (3–3.5 cm.). Weight, 0.17–0.21 ounces (5–6 grams) each. Left usually larger.

Brain. Weight, female 44–45 ounces (1250–1275 grams), male 48–51 ounces (1365–1450 grams).

Heart. Weight, female 8.8–9.8 ounces (250–280 grams), male 9.5–12.7 ounces (270–360 grams). Length, 4.7–5.9 inches (12–15 cm.). Breadth, 3.5–4.3 inches (9–11 cm.). Thickness, 2–3.1 inches (5–8 cm.). Thickness, wall left ventricle, 0.35–0.47 inch (9–12 mm.), right ventricle, 0.1–0.12 inch (2.5–3 mm.). Circumference, mitral orifice, 4.1–4.3 inches (10.4–10.9 cm.). Circumference, tricuspid orifice, 4.7–5 inches (12–12.7 cm.). Circumference, aortic orifice, 3–3.2 inches (7.7–8 cm.). Circumference, pulmonary orifice, 3.4–3.6 inches (8.5–9 cm.).

Intestines. Small intestine, length, 22.5 ft. (6.75 meters); ⅖ jejunum and ⅗ ileum. Diameter from 1.85 inches (47 mm.) in duodenum to 1.06 inches (27 mm.) at the end of ileum. Large intestine, length, 70.9–76.8 inches (180–195 cm.). Duodenum, length, 10.2–11.2 inches (26–28.5 cm.).

Kidneys. Weight, left, 5.3 ounces (150 grams), right, 5 ounces (140 grams). Thickness of cortex, 0.4 inch (1 cm.).

Liver. Weight 50–60 ounces (1440–1680 grams). Greatest transverse diameter, 7.9–9.5 inches (20–24 cm.). Greatest antero-posterior diameter, 3.9–5.9 inches (10–15 cm.).

Lungs. Weight, combined, 36–45 ounces (1020–1290 grams). Weight, male, right lung, 24 ounces (680 grams), left lung, 21 ounces (600 grams). Weight, female, right lung, 17 ounces (480 grams), left lung, 14.8 ounces (420 grams).

Ovaries. Combined weight, 0.25 ounces (7 grams). Right slightly larger.

Pancreas. Weight, quite variable, 2.1–4.8 ounces (60–135 grams).

Prostate. Weight, 0.8 ounce (22 grams).

Spleen. Weight, 5.5–6.9 ounces (155–195 grams).

Testes. Combined weight, 0.5–0.9 ounce (15–25 grams).

Thyroid. Transverse diameter, 2.4–2.8 inches (6–7 cm.). Height 1.2 inches (3 cm.). Weight, 1–1.4 ounces (30–40 gms.).

Uterus. (Virginal) length, 2.8 inches (7 cm.). Breadth, 1.6 inches (4 cm.). Thickness, 1 inch (2.5 cm.). Weight, 1.4–1.8 ounces (40–50 gm.). The dimensions of a multiparous uterus are each increased 1 cm. or more and the weight is increased 0.7 ounce (20 grams).

B. Physiological Normals.

Blood.

Specific gravity	1.041 to 1.067
Specific gravity (blood-serum)	1.026 to 1.032.
Total solids	20.0%
Hæmoglobin	14.0%
Serum albumin	4.52%
Paraglobulin	3.10%
Fibrinogen	0.42%
Glucose	0.084 to 0.10%
Sodium chloride	0.65%
Cholesterol	0.15%
Total Nitrogen	3.0%
Non-protein Nitrogen	25 to 35 mg. in 100 grams blood.
Urea Nitrogen	12 to 23 mg. in 100 grams blood.
Amino-acid Nitrogen	4 to 5 mg. in 100 grams blood.
Ammonia Nitrogen	0.1 to 0.2 mg. in 100 grams blood.
Uric acid	1 to 2 mg. in 100 grams blood.
Creatinine	1 to 2 mg. in 100 grams blood.
Creatine	5 to 9 mg. in 100 grams blood.
Acetone and aceto-acetic acid	0 to 1 mg. in 100 grams blood.
B hydroxy-butyric acid	0 to 3 mg. in 100 grams blood.
Hydrogen Ion concentration (oxalated blood)	7.4 to 7.6
Hydrogen Ion concentration (blood-serum)	7.6 to 7.8

Cerebro-spinal fluid.

Specific gravity	1.007 to 1.010.
Urea	0.01 to 0.05%.
Total proteid	0.025% (Albumin not present normally).
Pressure of fluid	5 to 7.5 mm. mercury or 60 to 100 mm water.

Fæces.

Amount in twenty-four hours on ordinary mixed diet, 110 to 170 grams (Solids 25 to 45 grams).

Amount in twenty-four hours on vegetable diet up to 350 grams (Solids 75 grams).

Average daily output, moist fæces (Hawk) 100 grams.

Gastric juice.

One hour after Ewald breakfast.

Quantity	40 to 50 c.c.
Total acidity	40 to 60 (0.15 to 0.22%).
Free HCl	20 to 60 (0.05 to 0.2%).
Combined	½ to 3 (0.01 to 0.1%).

Contents of fasting stomach (residuum) 20 to 50 c.c. (Rehfuss tube).

Total acidity of residuum	30
Free HCl acidity	19

Respiration.

Composition of alveolar air: Oxygen, 14.5%; Carbon dioxide, 5.5%; Nitrogen 80%.

Some give CO_2 alveolar content as from 3.7 to 5.5 volume per cent., or CO_2 tension of alveolar air as from 35 to 40 mm.

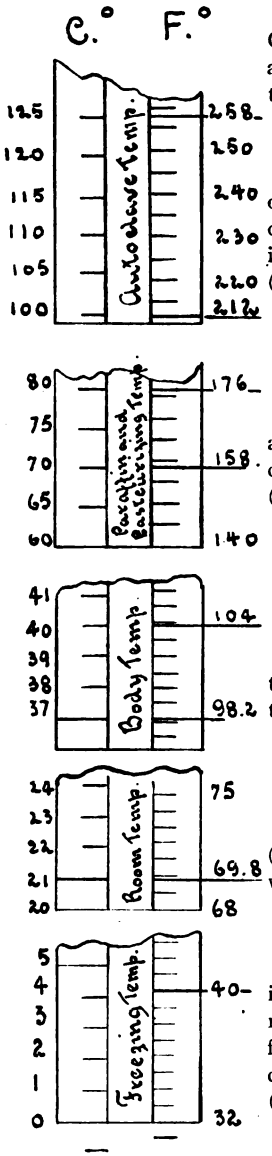
Air hunger in diabetes or chronic nephritis only begins when CO_2 tension has fallen to 20 or 25 mm.

Urine. Amount (American male) 1200 c.c.

Specific gravity	1.015 to 1.025
Urea	2.3% (35 grams in twenty-four hours) about 90% of total nitrogen.
Uric acid	0.05% (0.75 grams in twenty-four hours).
Creatinine	0.07% (1 gram in twenty-four hours).
Ammonia	0.04% (0.7 gram in twenty-four hours).
NaCl	1.1% (16.5 grams in twenty-four hours).

INDEX

Douglas Boyd Jr.
Harvard Medical School.
Boston.
or
430 W. Taylor St.
Griffin, Georgia.



Equivalent Fahrenheit and Centigrade tables for the temperatures in common use in laboratories:

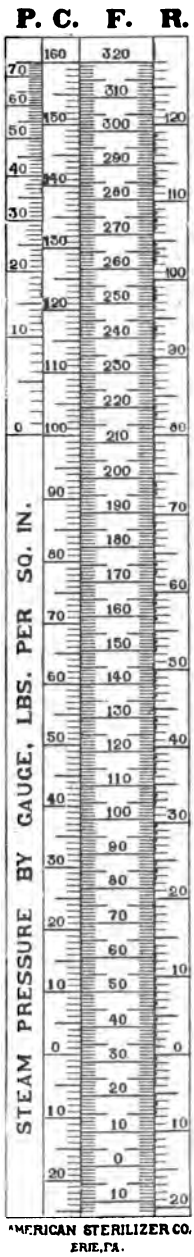
1. Those for sterilization of dressings and media. Also for certain disinfection of spore-bearing bacterial contamination (autoclave temperatures).

2. Those for pasteurization and sterilization of bacterial vaccines. Also for paraffin bath (pasteurizing temperatures).

3. Those for growing important pathogenic organisms (body temperatures).

4. Those for culturing gelatin (melting point, 25°C.) as in water work (room temperatures).

5. Those employed in preserving biological products and post-mortem material; also in centrifuging experiments to separate complement and amboceptor (freezing temperatures).



UNITS IN COMMON USE IN LABORATORIES

Cubic Meter.—Unit of space for the number of organisms in air. It contains 1000 liters. It is equal to 1.308 cubic yards or 35.316 cubic feet. One thousand cubic feet, the unit of space in disinfection, is equal to 28.3+ cubic meters. One cubic decimeter is one liter and equals 0.908 quarts dry measure or 1.0567 quarts liquid measure.

Liter.—Unit of space for normal volumetric solutions. It contains 1000 cubic centimeters. It is equal to 1.0567 quarts or 33.8+ ounces. A liter of distilled water weighs 1 kilogram. One U. S. gallon is equal to 3785 c.c. and one imperial gallon to 4543 c.c. One fluid ounce equals 29.57 c.c.

Cubic Centimeter.—Unit of space for organisms in water, milk, vaccines, etc., 1 c.c. = 0.27 fl. dr. There are, approximately, 20 drops in 1 c.c. of water, provided the capillary pipette has a bore of about 1 mm. and is held horizontally. A finely drawn capillary pipette, held vertically, will deliver about 50 drops from 1 c.c.

Cubic Millimeter.—Unit of space for blood-cells. There are 1000 cubic millimeters in 1 cubic centimeter and 1 million cubic millimeters in 1 liter. In water analysis, as there are 1 million milligrams in one liter, parts in the million and milligrams per liter are the same.

1 Meter = 39.37 inches.
1 Centimeter = 0.3937 inch. Approximately, 2/5 inch.
1 Millimeter = 0.0393 inch. Approximately, 1/25 inch.
1 Inch = 25.4 mm.
1 Yard = 0.9144 m.
1 Kilogram = 2.2+pounds av.
1 Gram = 15.432 grains.
1 Centigram = 0.154 grain.
1 Milligram = 0.0154 grain. Approximately, 1/64 grain.
A pound avoirdupois is equal to 453.59 gm.
1 Oz. avoirdupois is equal to 28.35 gm.

One hundred cubic centimeters of a saturated solution contains:

	Water	Alcohol
Methylene blue,	6.68	0.66 gram.
Gentian violet,	1.75	4.42 grams.
Basic fuchsin,	0.66	2.92 grams.

Key to Table on opposite page.

— = negative, + = positive, O = no change, A = acid, Alk. = alkaline, G = gas; Fl = fluorescence, Pep = peptonization, 1 = litmus Russell's medium not reduced; 2 = reduced, 3 = action variable. Proteus gives a peculiar dark, heavy oil-like fluorescence in neutral red glucose bouillon.

Important non-spore bearing Gram negative intestinal bacilli.	Capsules	Motility	Gelatine	Milk (coagulation)	Litmus Milk			Glucose	Maltose	Lactose	Mannite	Saccharose	Glucose neutral red bouillon	Russell's Medium		Indol	Voges-Proskauer	Remarks
					1st day	3d day	12th day							Butt	Slant			
1. B. fæcalis alkaligines.	–	+	–	–	Alk	Alk	Alk	O	O	O	O	O	O	O	O	–	–	Found in fæces and sewage-contaminated water. Differs from B. typhos. by marked alkali production.
2. B. typhosus.	–	+	–	–	A	A	A Alk	A	A	O	A	O	O	A	Alk	–	–	Blood cultures first week—agglutination afterward.
3. B. dysenteriæ (Shiga-Kruse).	–	–	–	–	A	Alk	Alk	A	O	O	O	O	O	A	Alk	–	–	Nonacid strain, highly toxic.
4. B. dysenteriæ (Flexner-Strong).	–	–	–	–	A	Alk	Alk	A	A	O	A	O	O	A	Alk	–	–	Acid mannite strain, moderate toxicity.
"Y."	–	–	–	–	A	Alk	Alk	A	O	O	A	O	O	A	Alk	±	–	Much like Flexner strain. No acid in maltose.
o. 1.	–	+	–	–	O	O	Alk	A G	O	O	O	O	G	.....	.....	+	–	Found in summer diarrhœa of children.
sus A.	–	+	–	–	A	A	A Alk	A G	A G	O	A G	O	G	A G	Alk	–	–	Little gas. No fluorescence n. red. Litmus milk acid in third day. 1.
sus B.	–	+	–	–	A	Alk	Alk	A G	A G	O	A G	O	G Fl	A G	Alk	–	–	Much gas. Marked reduction n. red with yellow fluorescence. Litmus milk alkaline third day. 2.
s. .)	–	+	–	–	A	Alk	Alk	A G	A G	O	A G	O	G Fl	A G	Alk	–	–	B. choleræ suis, B. icteroides, B. of Danysz virus and B. paratyphoid B. closely related (Gaertner group). 2.
	–	+	–	+	A	A	A	A G	A G	A G	A G	O	G Fl	A G	A	+	–	There is also a B. coli anærogenes which is like B. coli but does not form gas.
11. B. lactis ærogenes.	+	–	–	+	A	A	A	A G	A G	A G	A G	A G	G Fl	A G	A	±	+	Very nearly related to Friedlander's bacillus as well as to B. coli. 3.
12. B. cloacæ.	–	+	+	+	O	A	Pep.	A G	A G	A G	A G	A G	G Fl	A G	A	+	+	Differs from B. coli in liquefaction of gelatin and shows slow production of gas in lactose.
13. B. proteus vulgaris.	–	+	+	+	O	Alk	Pep.	A G	O	O	O	O	G Fl	A G	Alk	–	..	Three types—Proteus vulgaris rapid gealtin liq.; P. mirabilis slow gelatin liq. Spread- 2.

9.A.1918.2-A
Practical bacteriology, blood w1918
Countway Library AIV9324
3 2044 045 136 306